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(54) **TARGETING COMPLEMENT FACTOR H FOR TREATMENT OF DISEASES**

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(57) **ABSTRACT**

The invention provides a CR2-FH molecule comprising a CR2 portion comprising CR2 protein or a fragment thereof and a FH portion comprising a factor H protein or a fragment thereof, and pharmaceutical compositions comprising a CR2-FH molecule. Also provided are methods of using the compositions for treatment diseases in which the alternative complement pathway is implicated, such as age-related macular degeneration, rheumatoid arthritis, and ischemia reperfusion.

13 Claims, 31 Drawing Sheets

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Figure 1

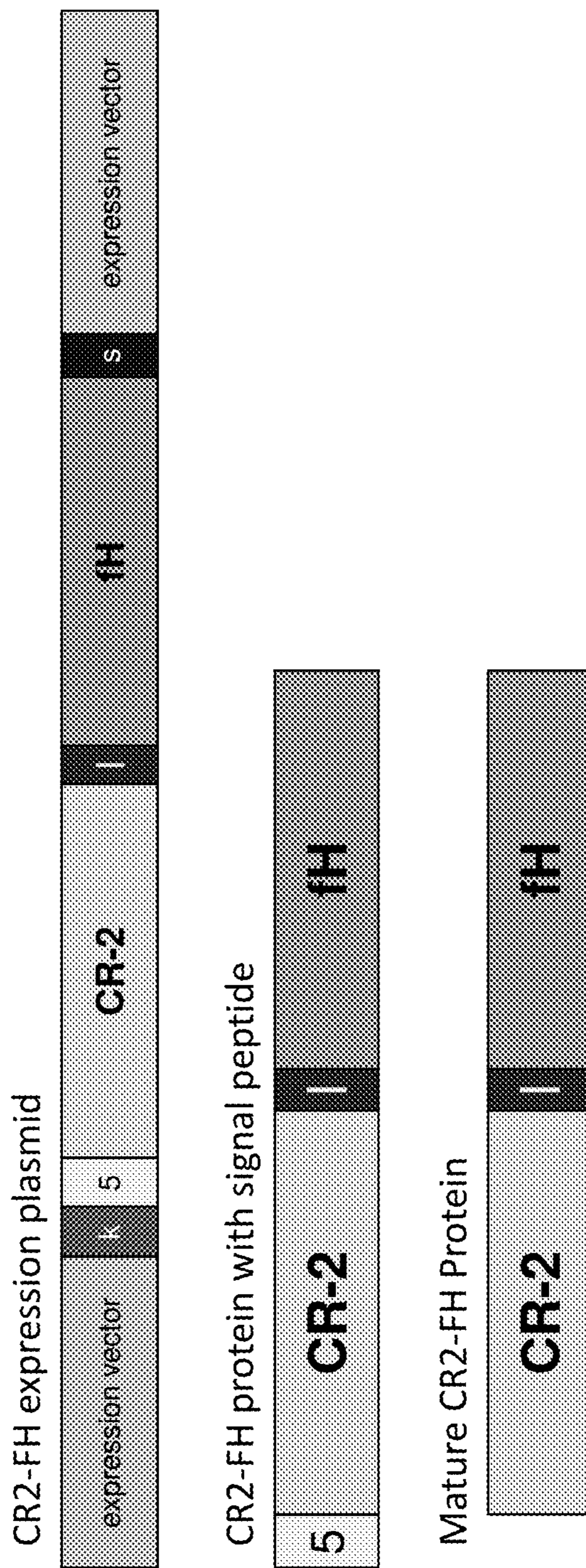


Figure 2

Amino acid sequence of human CR2 (SEQ ID NO:1)

MGAAGLLGVFLALVAPGVLGISCGSPPPILNGRISYYSTPIAVGTVIRYSCSGTFRLIGE
KSLLCITKDKVDGTWTKPAPKCEYFNKYSSCPEPIVPGGYKIRGSTPYRHGDSVTFAC
KTNFSMNGNKS VWCQANNMWGPTRLPTCVSVFPLECPALPMIHNGHHTSEN VGSIA
PGLSVTYSCESGYLLVGEKIINCLSSGKWSAVPPTCEEARKSLGRFPNGKVKEPPILR
VGV TANFFCDEGYRLQGPPSSRCVIAGQGVAVTKMPVCEEIFCPSPPPI LNGRHIGNS
LANVSYGSIVTYTCDPDPEEGVNFILIGESTLRCTVDSQKTGTWSGPAPRCELSTSAVQ
CPHPQILRGRMVSGQKDRYTYNDTVIFACMFGFTLK GSKQIRCNAQGTWEPSAPVCE
KECQAPPNILNGQKEDRHMVRFDPGTSIKYSCNPGYVLVGEESIQCTSEG VWTTPVPQ
CKVAACEATGRQLLTKPQHQFVRPDVNSSCGEGYKLSGSVYQECQGTIPWFMEIRLC
KEITCPPPPVIYNGAHTGSSLEDFPYGTTV TYTCNPGPERGVEFSLIGESTIRCTSNDQE
RGTWSGPAPLCKLSLLAVQC SHVHIANGYKISGKEAPYFYNDTVTFKCYSGFTLKGS
SQIRCKRDNTWDPEIPVCEKGCQPPPGLHHGRHTGGNTVFFVSGMTVDYTCDPGYLL
VGNKSIHCMPSGNWSPSAPRCEETCQHVRQSLQELPAGSRVELVNTSCQDGYQLTGH
AYQMCQDAENGIWFKKIPLCKVIHCHPPPVI VNGKHTGMMMAENFLYGNEVSYECDQ
GFYLLGEKNCSAEVILKAWILERA FPQCLRSLCPNPEVKHGYKLNKTHSA YSHNDIV
YVDCNPGFIMNGSRVIRCHTDNTWVPGVPTCIKKAFIGCPPPPKTPNGNHTGGNIARF
SPGMSILYSCDQGYLVVGEPLLLCTHEGTWSQ PAPHCKEVNCS SPADMDGIQKGLEP
RKMYQYGAVVTLECEDGYMLEGSPQSQCQSDHQWNPPLAVCRSRSLAPVLCGIAAG
LILLTFLIVITLYVISKHRERNYTDTSQKEAFHLEAREVYSVDPYNPAS

Amino acid sequence of human FH (SEQ ID NO:2)

MRLLAIIICLMLWAICVAEDCNELPPRRNTEILTGSWSDQTYPEGTQAIYKCRPGYRS
LGNVIMVCRKGEWVALNPLRKCQKRPCGHPGDTPFGTFTLTGGNVFEYGVKAVYTC
NEGYQLLGEINYRECDTDGWTNDIPICEVVKCLPVTAPENKIVSSAMEPDREYHFG
QAVRFVCNSGYKIEGDEEMHCSDDGFW SKEKPKCVEISCKSPDVINGSPISQKIIYKEN
ERFQYKCNMGYEYSERGDVCTESGWRPLPSCEEKSCDNPIYNGDY SPLRIKHRTG
DEITYQCRNGFY PATRGNTAKCTSTGWIPAPRCTLKPCDY PDIKHGGLYHENMRRPY
FPVAVGKYYSY CDEHFETPSGSYWDHIHCTQDGWSPA VPCLRKCYFPYLENGYNQ
NHGRKFVQGKSIDVACHPGYALPKAQT TVTCMENGWSPTPRCIRVK TCSKSSIDIEN
GFISESQYTYALKEKAKYQCKLGYVTADGETSGSIRCGKDGWSAQPTCIKSCDIPVF
MNARTKNDFTWFKLNDTLDYECHDGYESNTGSTTGSIVCGYNGWSDLPICYERECE
LPKIDVHLVPDRKKDQYKVGEVLKFSCKPGFTIVGPNSVQCYHFGLSPDLPICKEQVQ
SCGPPPELLNGNVKEKTKEEYGHSEVVEY YCNPRFLMKGPNKIQCVDGEWTTLPVCI
VEESTCGDIPELEHGWAQLSSPPYYYGDSVEFNCSSESFTMIGHRSITCIHGVWTQLPQ
CVAIDKLKCKSSNLIILEHLKNKKEFDHNSNIRYRCRGKEGWIHTVCINGRWDPEV
NCSMAQIQLCPPPPQIPNSHNMTTTLN YRDGEKVSVLCQENYLIQEGEEITCKDGRW
QSIPLC VEKIPCSQPPQIEHGTINSSRSSQESYAHGTKLSYTC EGGFRISEENETTCYMG
KWSSPPQCEGLPCKSPPEISHGVVAHMSDSYQY GEEVTYKCFEGFGIDGPAIAKCLGE
KWSHPPSCIKTDCLSLPSFENAIPMGEKKDVYKAGEQV TYTCATYYKMDGASNVTCI
NSRWTGRPTCRDTSCVNPPTVQNA YIVSRQMSKYPSGERVRYQCRSPYEMFGDEEV
MCLNGNWTEPPQCKDSTGKCGPPPIDNGDITSFPLSVYAPASSVEYQCQONLYQLEG
NKRITCRNGQWSEPPKCLHPCVISREIMENY NIALRWTAKQKLYSRTGESVEFVCKR
GYRLSSRSHTLR TTCWDGKLEYPTCAKR

Figure 3

Amino acid sequence of human CR2-FH (SEQ ID NO:3)

ISCGSPPPILNGRISYYSTPIAVGTVIRYSCSGTFRLLIGEKSLLCITKDKVDGTWDPAP
KCEYFNKYSSCPEPIVPGGYKIRGSTPYRHGDSVTFACKTNFSMNGNKSVMWCQANNI
NNMWGPTRLPTCVSVFPLECPALPMIHNGHHTSENVGSIAPGLSVTYSCESGYLLVGE
KIINCLSSGKWSAVPPTCEEAXCKSLGRFPNGKVKEPPILRVGVTANFFCDEGYRLQG
PPSSRCVIAGQGVAWTKMPVCGGGGSGGGGSCVAEDCNELPPRRNTEILTGSWSDQ
TYPEGTQAIYKCRPGYRSLGNVIMVCRKGEWVALNPLRKCQKRPCGHPGDTDFGTFT
LTGGNVFEYGVKAVYTCNEGYQLLGEINYRECDTDGWTNDIPICEVVKCLPVTAPEN
GKIVSSAMEPDREYHFGQAVRFVCNSGYKIEGDEEMHCSDDGFWSKEKPKCVEISCK
SPDVINGSPISQKIYKENERFQYKCNMGYEYSERGDVCTESGWRPLPSCEEKSCDN
PYIPNGDYSPLRIKHRTGDEITYQCRNGFYPATRGNTAKCTSTGWIPAPRCT

Nucleic acid sequence of human CR2-FH (SEQ ID NO:4)

ATTTCTTGTGGCTCTCCTCCGCCTATCCTAAATGGCCGGATTAGTTATTATTCTAC
CCCCATTGCTGTTGGTACCGTGATAAGGTACAGTTGTTTCAGGTACCTTCCGCCTCA
TTGGAGAAAAAGTCTATTATGCATAACTAAAGACAAAGTGGATGGAACCTGGG
ATAAACCTGCTCCTAAATGTGAATATTTCAATAAATATTCTTCTTGCCCTGAGCCC
ATAGTACCAGGAGGATACAAAATTAGAGGCTCTACACCCTACAGACATGGTGAT
TCTGTGACATTTGCCTGTAAAACCAACTTCTCCATGAACGGAAACAAGTCTGTTT
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GTGTAAGTGTTCCTCTCGAGTGTCCAGCACTTCCTATGATCCACAATGGACAT
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GAAGAAATACAGAAATCTGACAGGTTCCCTGGTCTGACCAAACATATCCAGAAG
GCACCCAGGCTATCTATAAATGCCGCCCTGGATATAGATCTCTTGGAAATGTAAT
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GAAAAGGCCCTGTGGACATCCTGGAGATACTCCTTTTGGTACTTTTACCCTTACA
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GGAAAATTGTCAGTAGTGCAATGGAACCAGATCGGGAATACCATTTTGGACAA
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CATTGTTTCAGACGATGGTTTTTGGAGTAAAGAGAAACCAAAGTGTGTGGAAATTT
CATGCAAATCCCCAGATGTTATAAATGGATCTCCTATATCTCAGAAGATTATTTAT
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AGAGGAGATGCTGTATGCACTGAATCTGGATGGCGTCCGTTGCCTTCATGTGAAG
AAAATCATGTGATAATCCTTATAATCCAAATGGTGACTACTCACCTTTAAGGAT
TAAACACAGAACTGGAGATGAAATCACGTACCAGTGTAGAAATGGTTTTTATCCT
GCAACCCGGGAAATACAGCCAAATGCACAAGTACTGGCTGGATACCTGCTCCG
AGATGTACCT

Figure 4

SEQ ID NO:5, nnn = optional linker

ISCGSPPPILNGRISYYSTPIAVGTVIRYSCSGTFRLIGEKSLLCITKDKVDGTWDPAP
KCEYFNKYSSCPEPIVPGGYKIRGSTPYRHGDSVTFACKTNFSMNGNKSVWCQANN
MWGPTRLPTCVSVFPLECPALPMIHNGHHTSENVGSIAPGLSVTYSCESGYLLVGEKII
NCLSSGKWSAVPPTCEEARCKSLGRFPNGKVKEPPILRVGVTANFFCDEGYRLQGPPS
SRCVIAGQGVAWTKMPVChnnCVAEDCNELPPRRNTEILTGSWSDQTYPEGTQAIYK
CRPGYRSLGNVIMVCRKGEWVALNPLRKCQKRPCGHPGDTPFGTFTLTGGNVFEYG
VKAVYTCNEGYQLLGEINYRECDTDGWTNDIPICEVVKCLPVTAPENGGKIVSSAMEP
DREYHFGQAVRFVCNSGYKIEGDEEMHCSDDGFWSKEKPKCVEISCKSPDVINGSPIS
QKIIYKENERFQYKCNMGYEYSERGDAVCTESGWRPLPSCEEKSCDNPYIPNGDYSP
LRIKHRTGDEITYQCRNGFYPATRGNTAKCTSTGWIPAPRCT

SEQ ID NO:6, nnn = optional linker

ISCGSPPPILNGRISYYSTPIAVGTVIRYSCSGTFRLIGEKSLLCITKDKVDGTWDPAP
KCEYFNKYSSCPEPIVPGGYKIRGSTPYRHGDSVTFACKTNFSMNGNKSVWCQANN
MWGPTRLPTCVSVFPLECPALPMIHNGHHTSENVGSIAPGLSVTYSCESGYLLVGEKII
NCLSSGKWSAVPPTCEEARCKSLGRFPNGKVKEPPILRVGVTANFFCDEGYRLQGPPS
SRCVIAGQGVAWTKMPVChnnCVAEDCNELPPRRNTEILTGSWSDQTYPEGTQAIYK
CRPGYRSLGNIIMVCRKGEWVALNPLRKCQKRPCGHPGDTPFGTFTLTGGNVFEYGV
KAVYTCNEGYQLLGEINYRECDTDGWTNDIPICEVVKCLPVTAPENGGKIVSSAMEPD
REYHFGQAVRFVCNSGYKIEGDEEMHCSDDGFWSKEKPKCVEISCKSPDVINGSPISQ
KIIYKENERFQYKCNMGYEYSERGDAVCTESGWRPLPSCEEKSCDNPYIPNGDYSP
IKHRTGDEITYQCRNGFYPATRGNTAKCTSTGWIPAPRCT

Figure 5

SEQ ID NO:7, nnn = optional linker

ISCGSPPPILNGRISYYSTPIAVGTVIRYSCSGTFRLIGEKSLLCITKDKVDGTWDKPAP
KCEYFNKYSSCPEPIVPGGYKIRGSTPYRHGDSVTFACKTNFSMNGNKS VWCQANNI
NNMWGPTRLPTCVSVFPLECPALPMIHNGHHTSENVGSIAPGLSVTYSCESGYLLVGE
KIINCLSSGKWSAVPPTCEEAXCKSLGRFPNGKVKEPPILRVGVTANFFCDEGYRLQG
PPSSRCVIAGQGVAWTKMPVC_{nnn}EDCNELPPRRNTEILTGSWSDQTYPEGTQAIYKC
RPGYRSLGNVIMVCRKGEWVALNPLRKCQKRPCGHPGDT_{nnn}PF_{nnn}FTLTGGNVFEYGV
KAVYTCNEGYQLLGEINYRECDTDGWTNDIPICEVVKCLPVTAPENGKIVSSAMEPD
REYHFGQAVRFVCNSGYKIEGDEEMHCSDDGFWSKEKPKCVEISCKSPDVINGSPISQ
KIIYKENERFQYKCNMGYEYSERGDAVCTESGWRPLPSCEEKSCDNPYIPNGDYSPLR
IKHRTGDEITYQCRNGFYPATRGNTAKCTSTGWIPAPRCT

SEQ ID NO:8, nnn = optional linker

ISCGSPPPILNGRISYYSTPIAVGTVIRYSCSGTFRLIGEKSLLCITKDKVDGTWDKPAP
KCEYFNKYSSCPEPIVPGGYKIRGSTPYRHGDSVTFACKTNFSMNGNKS VWCQANNI
NNMWGPTRLPTCVSVFPLECPALPMIHNGHHTSENVGSIAPGLSVTYSCESGYLLVGE
KIINCLSSGKWSAVPPTCEEAXCKSLGRFPNGKVKEPPILRVGVTANFFCDEGYRLQG
PPSSRCVIAGQGVAWTKMPVC_{nnn}EDCNELPPRRNTEILTGSWSDQTYPEGTQAIYKC
RPGYRSLGNIIIMVCRKGEWVALNPLRKCQKRPCGHPGDT_{nnn}PF_{nnn}FTLTGGNVFEYGVK
AVYTCNEGYQLLGEINYRECDTDGWTNDIPICEVVKCLPVTAPENGKIVSSAMEPDR
EYHFGQAVRFVCNSGYKIEGDEEMHCSDDGFWSKEKPKCVEISCKSPDVINGSPISQK
IYKENERFQYKCNMGYEYSERGDAVCTESGWRPLPSCEEKSCDNPYIPNGDYSPLRI
KHRTGDEITYQCRNGFYPATRGNTAKCTSTGWIPAPRCT

Figure 6

SEQ ID NO:9, nnn = optional linker

ISCGSPPPILNGRISYYSTPIAVGTVIRYSCSGTFRLIGEKSLLCITKDKVDGTWDPAP
KCEYFNKYSSCPEPIVPGGYKIRGSTPYRHGDSVTFACKTNFSMNGNKSVMWCQANN
MWGPTRLPTCVSVFPLECPALPMIHNGHHTSENVGSIAPGLSVTYSCESGYLLVGEKII
NCLSSGKWSAVPPTCEEARKSLGRFPNGKVKEPPILRVGVTANFFCDEGYRLQGPPS
SRCVIAGQGVAWTKMPVC_{nnn}EDCNELPPRRNTEILTGSWSDQTYPEGTQAIYKCRPG
YRSLGNVIMVCRKGEWVALNPLRKCQKRPCGHPGDT_{nnn}PF_{nnn}GTFTLTGGNVFEYGVKAV
YTCNEGYQLLGEINYRECDTDGWTNDIPICEVVKCLPVTAPENGKIVSSAMEPDREY
HFGQAVRFVCNSGYKIEGDEEMHCSDDGFWSKEKPKCVEISCKSPDVINGSPISQKIIY
KENERFQYKCNMGYEYSERGDAVCTESGWRPLPSCEEKSCDNPYIPNGDYSPLRIKH
RTGDEITYQCRNGFY_{nnn}PATRGNTAKCTSTGWIPAPRCT

SEQ ID NO:10, nnn = optional linker

ISCGSPPPILNGRISYYSTPIAVGTVIRYSCSGTFRLIGEKSLLCITKDKVDGTWDPAP
KCEYFNKYSSCPEPIVPGGYKIRGSTPYRHGDSVTFACKTNFSMNGNKSVMWCQANN
MWGPTRLPTCVSVFPLECPALPMIHNGHHTSENVGSIAPGLSVTYSCESGYLLVGEKII
NCLSSGKWSAVPPTCEEARKSLGRFPNGKVKEPPILRVGVTANFFCDEGYRLQGPPS
SRCVIAGQGVAWTKMPVC_{nnn}EDCNELPPRRNTEILTGSWSDQTYPEGTQAIYKCRPG
YRSLGNIIMVCRKGEWVALNPLRKCQKRPCGHPGDT_{nnn}PF_{nnn}GTFTLTGGNVFEYGVKAV
YTCNEGYQLLGEINYRECDTDGWTNDIPICEVVKCLPVTAPENGKIVSSAMEPDREY
HFGQAVRFVCNSGYKIEGDEEMHCSDDGFWSKEKPKCVEISCKSPDVINGSPISQKIIY
KENERFQYKCNMGYEYSERGDAVCTESGWRPLPSCEEKSCDNPYIPNGDYSPLRIKH
RTGDEITYQCRNGFY_{nnn}PATRGNTAKCTSTGWIPAPRCT

Figure 7

CD5 peptide sequence (SEQ ID NO:11)

MPMGSLQPLATLYLLGMLVAS

CD5 nucleotide sequence (SEQ ID NO:12)

ATGCCCATGGGGTCTCTGCAACCGCTGGCCACCTTGTACCTGCTGGGGATGCTGG
TCGCTTCCTGCCTCGGA

CR2 peptide sequence (SEQ ID NO:13)

MGAAGLLGVFLALVAPG

CR2 nucleotide sequence (SEQ ID NO:14)

ATGGGCGCCGCGGGCCTGCTCGGGGTTTTCTTGGCTCTCGTCGCACCGGGGGTCC
TCGGG

CR2 peptide sequence (SEQ ID NO:25)

MGAAGLLGVFLALVAPGVLG

CR2 nucleotide sequence (SEQ ID NO:26)

ATGGGAGCCGCTGGTCTGCTCGGCGTGTTCCTCGCCTTGGTGGCACCTGGCGTCC
TGGGC

Figure 8

Mouse CR2 amino acid sequence (SEQ ID NO:15)

MLTWFLFYFSEISCDPPPEVKNARKPY YSLPIVPGTVLRYTCSPSYRLIGEKAIFCISEN
QVHATWDKAPPICESVNKTISCSDPVPGGFMNKGSKAPFRHGDSVTFTCKANFTMK
GSKTVWCQANEMWGPTALPVCESDFPLECP SLPTIHNGHHTGQHVDQFVAGLSVTY
SCEPGYLLTGKKTIKCLSSGDWDGVIPTCKEAQCEHPGKFPNGQVKEPLSLQVGTTV
YFSCNEGYQLQGQPSSQCVIVEQKAIWTKKPVCKEILCPPPPPVRNGSHTGSFSENV
YGSTVTYTCDPSPKGVSTLIGEK TINCTTGSQKTGIWSGPAPYCVLSTSAVLCLQPK
IKRGQILSILKDSYSYNDTVAFSCEPGFTLKG NRSIRCNAHGTWEPPVPVCEKGCQAPP
KIINGQKEDSYLLNFDPGTSIRYSCDPGYLLVGEDTIHCTPEGKWTPI TPQCTVAECKP
VGPHLFRPQNQFIRTAVNSSCDEGFQ LSEAYQLCQGTIPWFIEIRLCKEITCPPPPVI
HNGTHTWSSSEDVPYGT VVTYMCYPGPEEGVKFKLIGEQT I HCTSDSRGRGSWSSPA
PLCKLSLPAVQCTDVHVENGVKLTDNKAPYFYND SVMFKCDDGYILSGSSQIRCKAN
NTWDPEKPLCKKEGCEPMRVHGLPDDSHIKLVKRTCQNGYQLTGYTYEKQNAEN
GTWFKKIEVCTVILCQPPPKIANGGHTGMMAKHFLYGN EVSYECDEGFYLLGEKSLQ
CVNDSKGHGSWSGPPPQCLQSSPLTHCPDPEVKHG YKLNKTHSAF SHNDIVHFVCNQ
GFIMNGSHLIRCHTNNTWLPGVPTCIRKASLGCQSPSTIPNGNHTGGSIARFPPGMSV
MYSCYQGFLMAGEARLIC THEGTWSQPPPFCKE VNCSFPEDTNGIQKGFQPGKTYRF
GATVTLECEDGYTLEGSPQSQCQDDSQWNPP LALCKYRRWSTIPLICGISVGSALILM
SVGFCMILKHRESNYTKTRPKEGALHLETRE VYSIDPYNPAS

Mouse FH amino acid sequence (SEQ ID NO:16)

MRLSARIHWLILWTVCAAEDCKGPPPRENSEILSGSWSEQLYPEGTQATYKCRPGYRT
LGTIVKVCKNGKWVASNPSRICRKKPCGHPGDTPFGSFRLAVGSQFEFGAKVVYTCD
DGYQLLGEIDYRECGADGWINDIPLCEVVKCLP VTELENGRIVSGAAETDQEY YFGQ
VVRFECSNGFKIEGHKEIHCSENGLWSNEKPRC VEILCTPPRVENGDGINVKPVYKEN
ERYHYKCKHGYVPKERGDAVCTGSGWSSQPFC EEKRCSPPYILNGIYTPHRIIHRSD
EIRYECNYGFYPVTGSTVSKCTPTGWIPVPRCT LKPCFEFPQFKYGRLYYEESLRPNFPV
SIGNKYSYKCDNGFSPPSGYSWDYLRCTA QGWEPEVPCVRKCVFHYVENGD SA YWE
KVYVQGQSLKVQCYNGYSLQNGQDTMTCTEN GWSPPPCKIRIKTCSASDIHIDNGFL
SESSSIYALNRETSYRCKQGYVTNTGEISGSIT CLQNGWSPQPSICKSCDMPVFENSITK
NTRTWFKLNDKLDYECLVGFENEYKHTKGSITCT YYGWSDTPSCYEREC SVPTLDRK
LVVSPRKEKYRVGDLLEFSCHSGHRVGPDSVQCY HFGWSPGFPTCKGQVASCAPPLE
ILNGEINGAKKVEYSHGEVVKYDCKPRFL LKGPNKIQCV DGNWTTLPVCIEEERTCG
DIPELEHGS AKCSVPPYHHGDSVEFICEENFTMIGHGSV SCISGKWTQLPKCVATDQL
EKCRVLKSTGIEAIKPKLTFETHNSTMDYKCRDKQE YERSICINGKWDPEPNCTSKTS
CPPPPQIPNTQVIETTVKYLDGEKLSVLCQDNYLTQD SEEMVCKDGRWQSLPRCIEKI
PCSQPPTIEHGSINLPRSSEERRDSIESSSHEHGTTF SYVCDDGFRIPEENRITCYMGKW
STPPRCVGLPCGPPPSIPLGTVSLELESYQHGE E VTYHCSTGFGIDGPAFIICEGGKWS
PPKCIKTDCDVLPTVKNAIIRGKSKKSYRTGEQVTFRCQSPYQMNGSDTVTCVNSRWI
GQPVCNDNSCVDPPHVPNATIVTRTKNKYLHGDRVRYECNKPLELFGQVEVMCENG
IWTEKPKCRGL*FDLSLKPSNVFSLDSTGKCGPPP IDNGDITSLSLPVYEPLSSVEYQC
QKY YLLKGGKTTTCTNGKWSEPTCLHACVIPENIMESHNIILKWRHTEKIYSHSGEDI
EFGCKYGYKARDSPPFRKTCINGTINYPTCV

Figure 9

SEQ ID NO:17 (MOUSE CR2-FH)

ISCDPPPEVKNARKPYYSPLIVPGTVLRYTCSPSYRLIGEKAIFCISENQVHATWDKAP
PICESVNKTISCSDPIVPGGFMNKGSKAPFRHGDSVTFTCKANFTMKGSKTVWCQAN
EMWGPTALPVCESDPFLCPSLPTIHNGHHTGQHVDQFVAGLSVTYSCEPGYLLTGK
KTIKCLSSGDWDGVIPTCKEAQCEHPGKFPNGQVKEPLSLQVGTTVYFSCNEGYQLQ
GQPSSQCVIVEQKAIWTKKPVCKEILEDCKGPPPRENSEILSGSWSEQLYPEGTQATY
KCRPGYRTLGTIVKVCKNGKWVASNPSRICRKKPCGHPGDTPFGSFRLAVGSQFEFG
AKVVYTCDDGYQLLGEIDYRECGADGWINDIPLCEVVKCLPVTLENGRIVSGAAET
DQEYYFGQVVRFECSNGFKIEGHKEIHCSENGLWSNEKPRCVEILCTPPRVENGDGIN
VKPVYKENERYHYKCKHGYVPKERGDAVCTGSGWSSQPFCCEEKRCSPPYILNGIYTP
HRIIHRSDDEIRYECNYGFYPVTGSTVSKCTPTGWIPVPRCT

SEQ ID NO:18 (MOUSE CR2-FH DNA)

ATGCCCATGGGGTCTCTGCAACCGCTGGCCACCTTGTACCTGCTGGGGATGCTGG
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AAACCTATTATTCTCTTCCCATAGTTCCTGGAAGTCTGAGGTACACTTGTTTC
ACCTAGCTACCGCCTCATTGGAGAAAAGGCTATCTTTTGTATAAGTGAAAATCAA
GTGCATGCCACCTGGGATAAAGCTCCTCCTATATGTGAATCTGTGAATAAAACCA
TTTCTTGCTCAGATCCCATAGTACCAGGGGGATTTCATGAATAAAGGATCTAAGGC
ACCATTACAGACATGGTGATTCTGTGACATTTACCTGTAAAGCCAACCTCACCATG
AAAGGAAGCAAACTGTCTGGTGCCAGGCAAATGAAATGTGGGGACCAACAGCT
CTGCCAGTCTGTGAGAGTGATTTCCCTCTGGAGTGCCCATCACTTCCAACGATTCA
TAATGGACACCACACAGGACAGCATGTTGACCAGTTTGTGCGGGGTTGTCTGTG
ACATACAGTTGTGAACCTGGCTATTTGCTCACTGGAAAAAAGACAATTAAGTGCT
TATCTTCAGGAGACTGGGATGGTGTCATCCCGACATGCAAAGAGGCCAGTGTA
ACATCCAGGAAAGTTTCCCAATGGGCAGGTAAAGGAACCTCTGAGCCTTCAGGTT
GGCACAACCTGTGTACTTCTCCTGTAATGAAGGGTACCAATTACAAGGACAACCCT
CTAGTCAGTGTGTAATTGTTGAACAGAAAGCCATCTGGACTAAGAAGCCAGTATG
TAAAGAAATTCTCGAAGATTGTAAAGGTCTCCTCCAAGAGAAAATTCAGAAATT
CTCTCAGGCTCGTGGTCAGAACTATATCCAGAAGGCACCCAGGCTACCTACA
AATGCCGCCCTGGATAACCGAACACTTGGCACTATTGTA AAAAGTATGCAAGAATGG
AAAATGGGTGGCGTCTAACCCATCCAGGATATGTCGGAAAAAGCCTTGTGGGCA
TCCCGGAGACACACCCTTTGGGTCTTTAGGCTGGCAGTTGGATCTCAATTTGAG
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AGTTGTGAAGTGTCTACCTGTGACAGA ACTCGAGAATGGAAGAATTGTGAGTGGT
GCAGCAGAAACAGACCAGGAATACTATTTTGGACAGGTGGTGCGGTTTGAATGC
AATTCAGGCTTCAAGATTGAAGGACATAAGGAAATTCATTGCTCAGAAAATGGC
CTTTGGAGCAATGAAAAGCCACGATGTGTGGAAATTCTCTGCACACCACCGCGAG
TGGAAAATGGAGATGGTATAAATGTGAAACCAGTTTACAAGGAGAATGAAAGAT
ACCACTATAAGTGTAAGCATGGTTATGTGCCCAAAGAAAGAGGGGATGCCGTCT
GCACAGGCTCTGGATGGAGTTCTCAGCCTTTCTGTGAAGAAAAGAGATGCTCACC
TCCTTATATTCTAAATGGTATCTACACACCTCACAGGATTATACACAGAAGTGAT
GATGAAATCAGATATGAATGTAATTATGGCTTCTATCCTGTA ACTGGATCAACTG
TTTCAAAGTGTACACCCACTGGCTGGATCCCTGTTCCAAGATGTACCT

Figure 10

GAATTCGCCGCCACCATGCCCATGGGGTCTCTGCAACCGCTGGCCACCTTGTACC
TGCTGGGGATGCTGGTCGCTTCCGTGCTAGCGATTTCTTGTGACCCTCCTCCTGAA
GTCAAAAATGCTCGGAAACCTATTATTCTCTTCCCATAGTTCCCTGGAACCTGTTCT
GAGGTACACTTGTTCACCTAGCTACCGCCTCATTGGAGAAAAGGCTATCTTTTGT
ATAAGTGAAAATCAAGTGCATGCCACCTGGGATAAAGCTCCTCCTATATGTGAAT
CTGTGAATAAAAACCAATTTCTTTGCTCAGATCCCATAGTACCAGGGGGATTTCATGAA
TAAAGGATCTAAGGCACCATTCAGACATGGTGATTCTGTGACATTTACCTGTAAA
GCCAACTTCACCATGAAAGGAAGCAAACTGTCTGGTGCCAGGCAAATGAAATG
TGGGGACCAACAGCTCTGCCAGTCTGTGAGAGTGATTTCCCTCTGGAGTGCCCAT
CACTTCCAACGATTCATAATGGACACCACACAGGACAGCATGTTGACCAGTTTGT
TGCGGGGTTGTCTGTGACATAACAGTTGTGAACCTGGCTATTTGCTCACTGGAAAA
AAGACAATTAAGTGCCTTATCTTCAGGAGACTGGGATGGTGTTCATCCCGACATGCA
AAGAGGCCCAGTGTGAACATCCAGGAAAGTTTCCCAATGGGCAGGTAAAGGAAC
CTCTGAGCCTTCAGGTTGGCACAACCTGTGTACTTCTCCTGTAATGAAGGGTACCA
ATTACAAGGACAACCCTCTAGTCAGTGTGTAATTGTTGAACAGAAAGCCATCTGG
ACTAAGAAGCCAGTATGTAAAGAAATTCTCGAAGATTGTAAAGGTCCTCCTCCAA
GAGAAAATTCAGAAATTCTCTCAGGCTCGTGGTCAGAACAACCTATATCCAGAAG
GCACCCAGGCTACCTACAAATGCCGCCCTGGATACCGAACACTTGGCACTATTGT
AAAAGTATGCAAGAATGGAAAATGGGTGGCGTCTAACCCATCCAGGATATGTCCG
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GTTGGATCTCAATTTGAGTTTGGTGCAAAGGTTGTTTATACCTGTGATGATGGGTA
TCAACTATTAGGTGAAATTGATTACCGTGAATGTGGTGACAGATGGCTGGATCAAT
GATATTCACCTATGTGAAGTTGTGAAGTGTCTACCTGTGACAGAACTCGAGAATG
GAAGAATTGTGAGTGGTGCAGCAGAAACAGACCAGGAATACTATTTTGGACAGG
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TTGCTCAGAAAATGGCCTTTGGAGCAATGAAAAGCCACGATGTGTGGAAATTCTC
TGCACACCACCGCGAGTGGAAAATGGAGATGGTATAAATGTGAAACCAGTTTAC
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TATACACAGAAGTGATGATGAAATCAGATATGAATGTAATTATGGCTTCTATCCT
GTAACCTGGATCAACTGTTTCAAAGTGTACACCCACTGGCTGGATCCCTGTTCCAA
GATGTACCGAAGATTGTAAAGGTCCTCCTCCAAGAGAAAATTCAGAAATTCTCTC
AGGCTCGTGGTCAGAACAACCTATATCCAGAAGGCACCCAGGCTACCTACAAATG
CCGCCCTGGATACCGAACACTTGGCACTATTGTAAAAGTATGCAAGAATGGAAA
ATGGGTGGCGTCTAACCCATCCAGGATATGTCCGAAAAAGCCTTGTGGGCATCCC
GGAGACACACCCTTTGGGTCCCTTTAGGCTGGCAGTTGGATCTCAATTTGAGTTTG
GTGCAAAGGTTGTTTATACCTGTGATGATGGGTATCAACTATTAGGTGAAATTGA
TTACCGTGAATGTGGTGCAGATGGCTGGATCAATGATATTCACCTATGTGAAGTT
GTGAAGTGTCTACCTGTGACAGAACTCGAGAATGGAAGAATTGTGAGTGGTGCA
GCAGAAACAGACCAGGAATACTATTTTGGACAGGTGGTGGCGGTTTGAATGCAATT
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GAGCAATGAAAAGCCACGATGTGTGGAAATTCTCTGCACACCACCGCGAGTGGA
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CTATAAGTGTAAAGCATGGTTATGTGCCCAAAGAAAGAGGGGATGCCGTCTGCAC
AGGCTCTGGATGGAGTTCTCAGCCTTTCTGTGAAGAAAAGAGATGCTCACCTCCT
TATATTCTAAATGGTATCTACACACCTCACAGGATTATACACAGAAGTGATGATG
AAATCAGATATGAATGTAATTATGGCTTCTATCCTGTAACCTGGATCAACTGTTTCA
AAGTGTACACCCACTGGCTGGATCCCTGTTCCAAGATGTACCTAA

Figure 11

GAATTCGCCGCCACCATGCCCATGGGGTCTCTGCAACCGCTGGCCACCTTGTACC
TGCTGGGGATGCTGGTCGCTTCCGTGCTAGCGATTTCTTGTGACCCTCCTCCTGAA
GTCAAAAATGCTCGGAAACCTATTATTCTCTTCCCATAGTTCCTGGAACCTGTTCT
GAGGTACACTTGTTCACCTAGCTACCGCCTCATTGGAGAAAAGGCTATCTTTTGT
ATAAGTGAAAATCAAGTGCATGCCACCTGGGATAAAGCTCCTCCTATATGTGAAT
CTGTGAATAAAACCATTTCTTGCTCAGATCCCATAGTACCAGGGGGATTTCATGAA
TAAAGGATCTAAGGCACCATTCAGACATGGTGATTCTGTGACATTTACCTGTAAA
GCCAACTTCACCATGAAAGGAAGCAAACTGTCTGGTGCCAGGCAAATGAAATG
TGGGGACCAACAGCTCTGCCAGTCTGTGAGAGTGATTTCCCTCTGGAGTGCCCAT
CACTTCCAACGATTCATAATGGACACCACACAGGACAGCATGTTGACCAGTTTGT
TGCGGGGTGTCTGTGACATAACAGTTGTGAACCTGGCTATTTGCTCACTGGAAAA
AAGACAATTAAGTGCTTATCTTCAGGAGACTGGGATGGTGTCATCCCGACATGCA
AAGAGGCCAGTGTGAACATCCAGGAAAGTTTCCCAATGGGCAGGTAAAGGAAC
CTCTGAGCCTTCAGGTTGGCACAACCTGTGTACTTCTCCTGTAATGAAGGGTACCA
ATTACAAGGACAACCCTCTAGTCAGTGTGTAATTGTTGAACAGAAAGCCATCTGG
ACTAAGAAGCCAGTATGTAAAGAAATTCTCGGCGGAGGTGGGTCCGGTGGCGGC
GGATCTGAAGATTGTAAAGGTCTCCTCCAAGAGAAAATTCAGAAATTCTCTCAG
GCTCGTGGTCAGAACAACCTATATCCAGAAGGCACCCAGGCTACCTACAAATGCC
GCCCTGGATACCGAACACTTGGCACTATTGTAAAAGTATGCAAGAATGGAAAAT
GGGTGGCGTCTAACCCATCCAGGATATGTCCGAAAAAGCCTTGTGGGCATCCCGG
AGACACACCCTTTGGGTCCCTTAGGCTGGCAGTTGGATCTCAATTTGAGTTTGGTG
CAAAGGTTGTTTATACCTGTGATGATGGGTATCAACTATTAGGTGAAATTGATTA
CCGTGAATGTGGTGCAGATGGCTGGATCAATGATATTCCACTATGTGAAGTTGTG
AAGTGTCTACCTGTGACAGAACCTCGAGAATGGAAGAATTGTGAGTGGTGCAGCA
GAAACAGACCAGGAATACTATTTTGGACAGGTGGTGCGGTTTGAATGCAATTCAG
GCTTCAAGATTGAAGGACATAAGGAAATTCATTGCTCAGAAAATGGCCTTTGGAG
CAATGAAAAGCCACGATGTGTGGAAATTCTCTGCACACCACCGCGAGTGGAAAA
TGGAGATGGTATAAATGTGAAACCAGTTTACAAGGAGAATGAAAGATACCACTA
TAAGTGTAAGCATGGTTATGTGCCCAAAGAAAGAGGGGATGCCGTCTGCACAGG
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ATTCTAAATGGTATCTACACACCTCACAGGATTATACACAGAAGTGATGATGAAA
TCAGATATGAATGTAATTTATGGCTTCTATCCTGTAACCTGGATCAACTGTTTCAAAG
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CTCCTCCAAGAGAAAAATTCAGAAATTCCTCAGGCTCGTGGTCAGAACAACCTATA
TCCAGAAGGCACCCAGGCTACCTACAAATGCCGCCCTGGATACCGAACACTTGGC
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ATATGTCGGAAAAAGCCTTGTGGGCATCCCGGAGACACACCCTTTGGGTCCCTTA
GGCTGGCAGTTGGATCTCAATTTGAGTTTGGTGCAAAGGTTGTTTATACCTGTGAT
GATGGGTATCAACTATTAGGTGAAATTGATTACCGTGAATGTGGTGCAGATGGCT
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CGAGAATGGAAGAATTGTGAGTGGTGCAGCAGAAACAGACCAGGAATACTATTT
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GGAAATTCCTCTGCACACCACCGCGAGTGGAAAATGGAGATGGTATAAATGTGAA
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CCTCACAGGATTATACACAGAAGTGATGATGAAATCAGATATGAATGTAATTATG
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CCTGTTCCAAGATGTACCTAA

Figure 12A

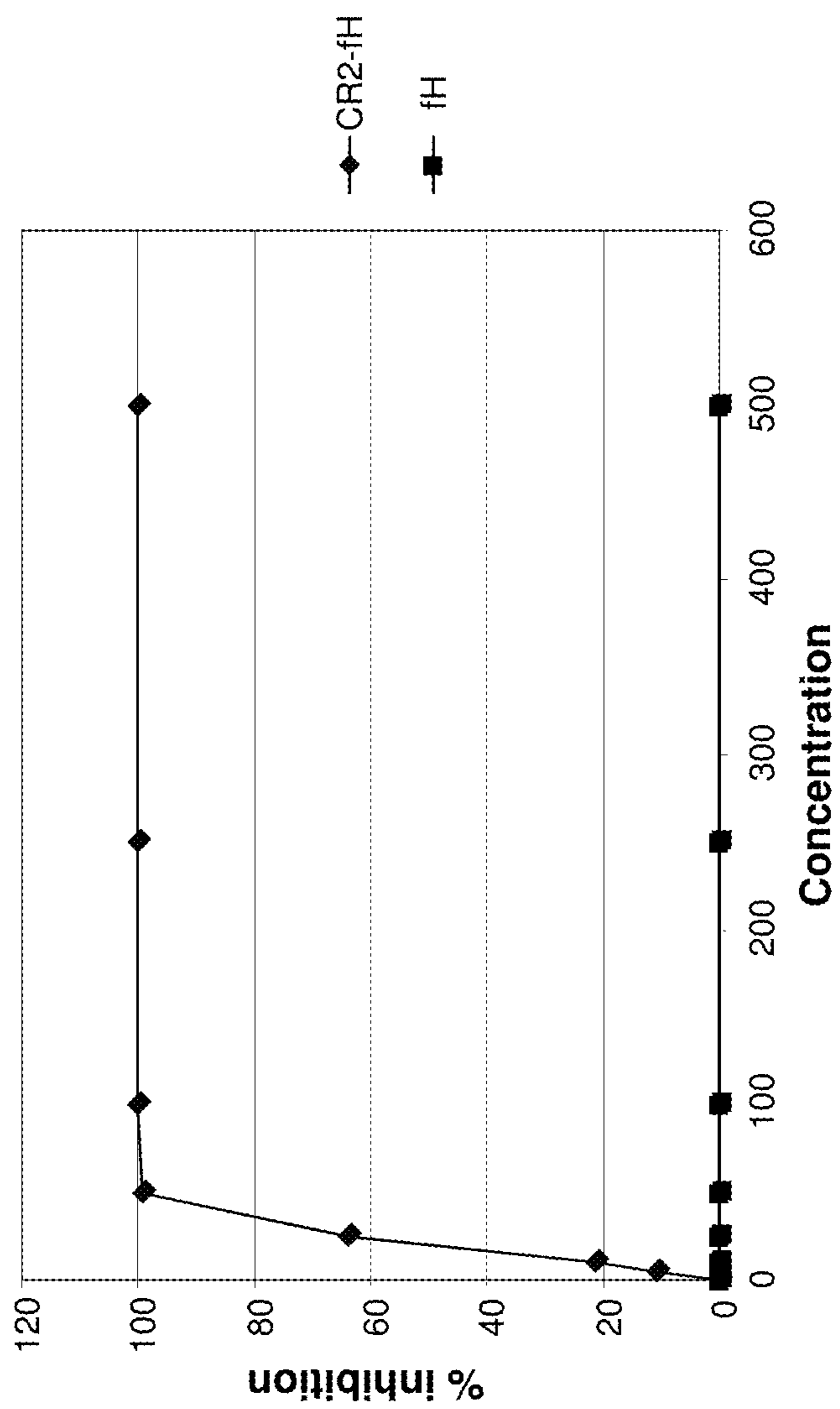


Figure 12B

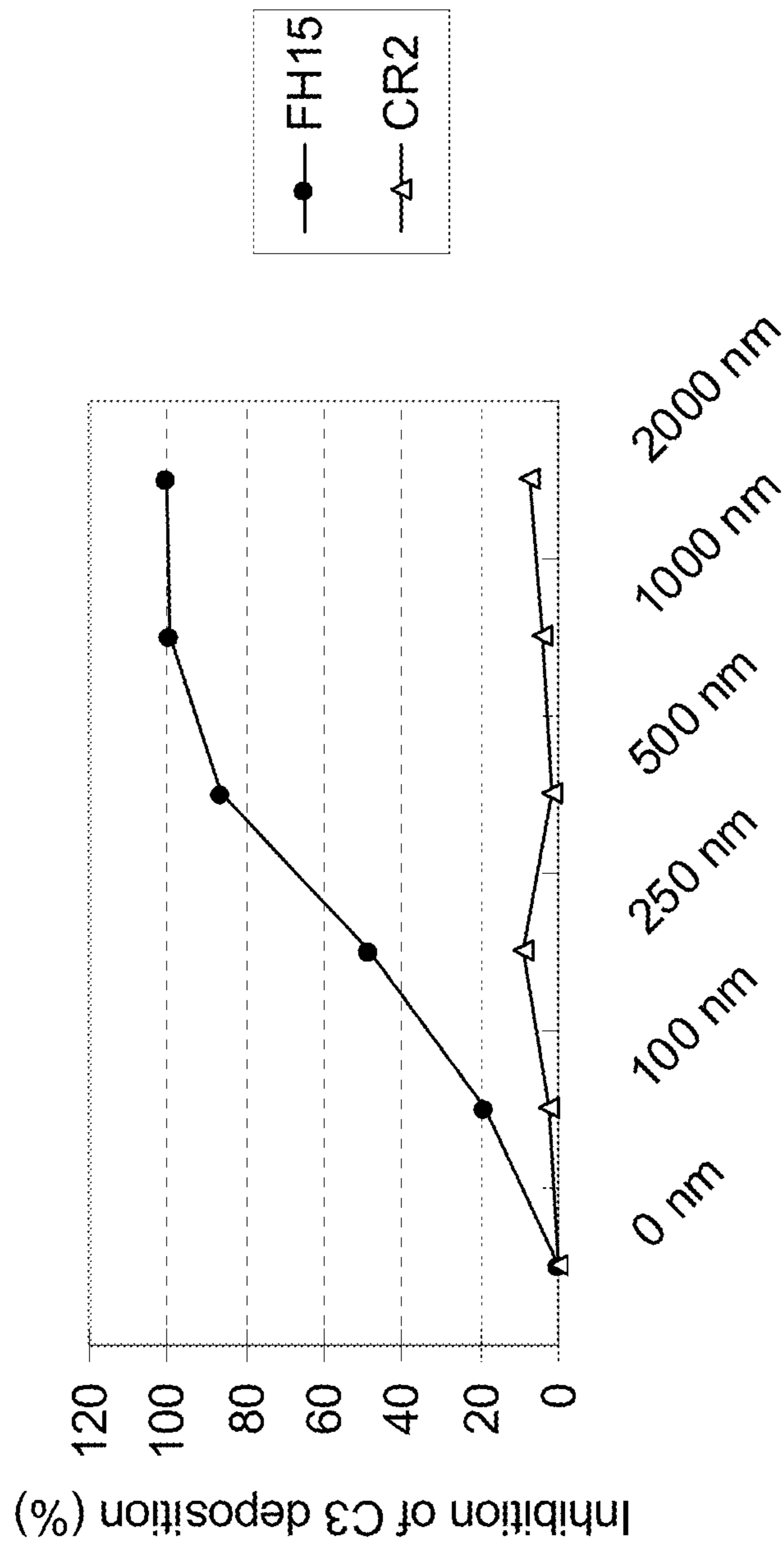


Figure 13

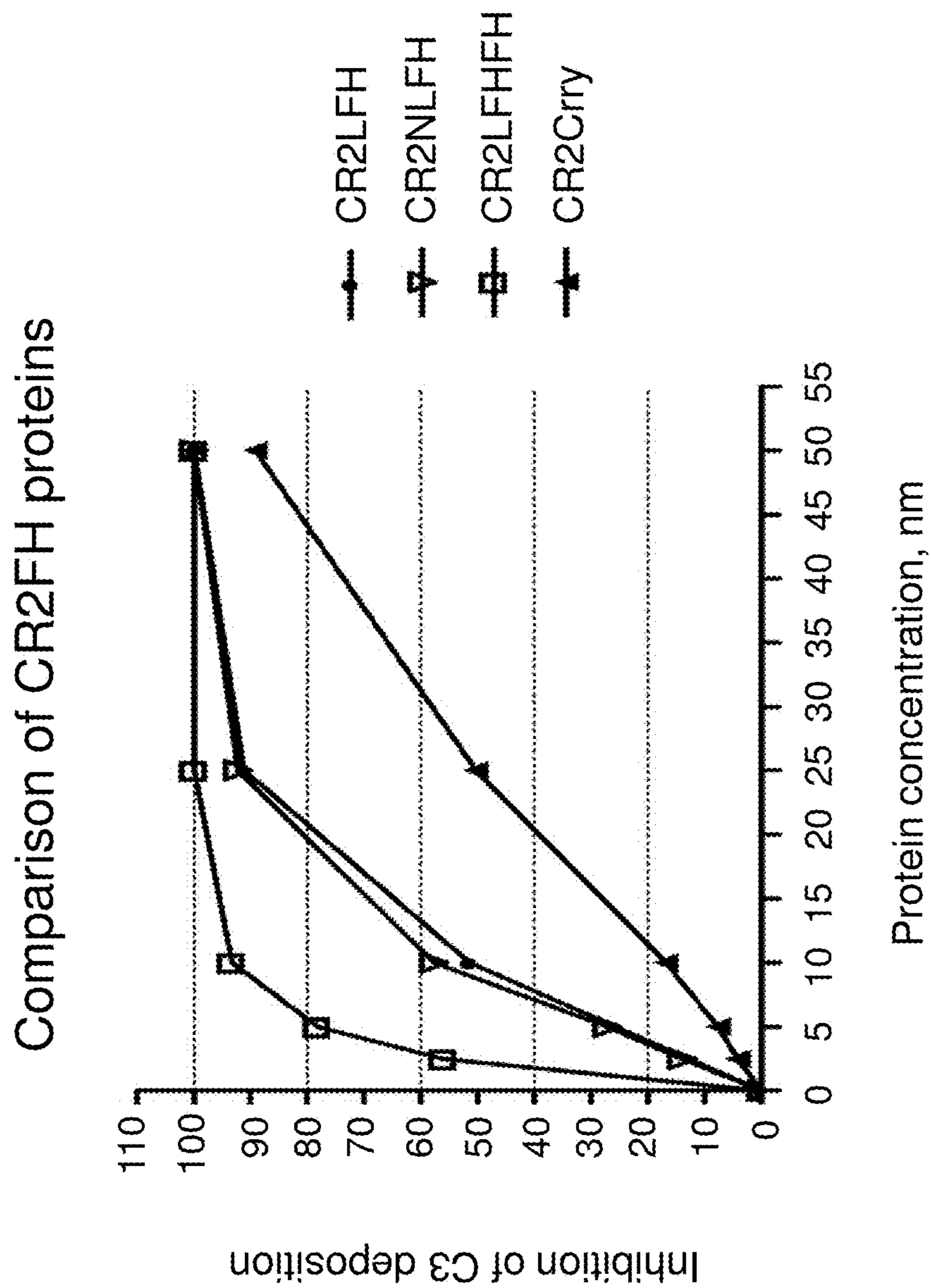


Figure 14A

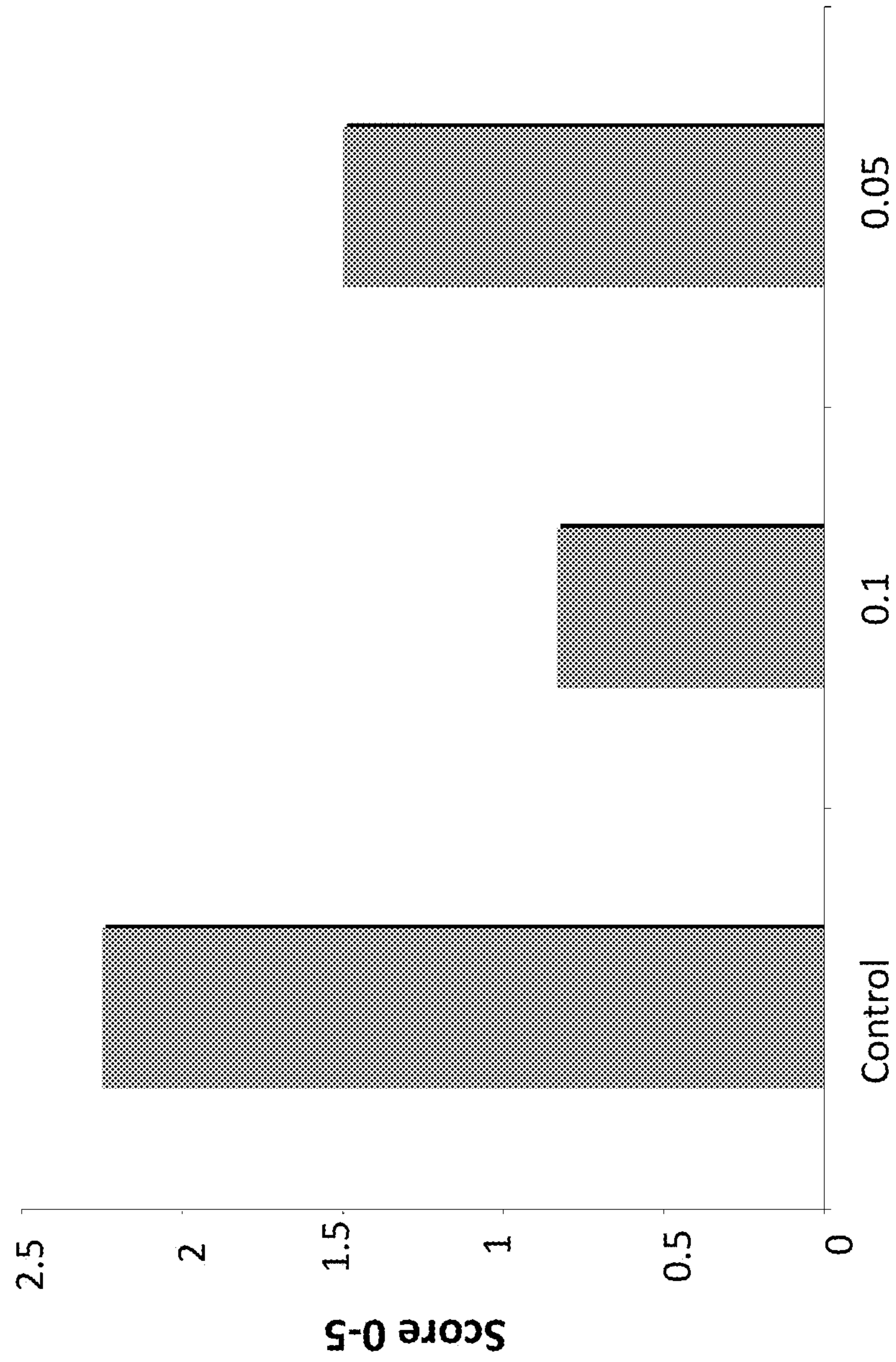


Figure 14B

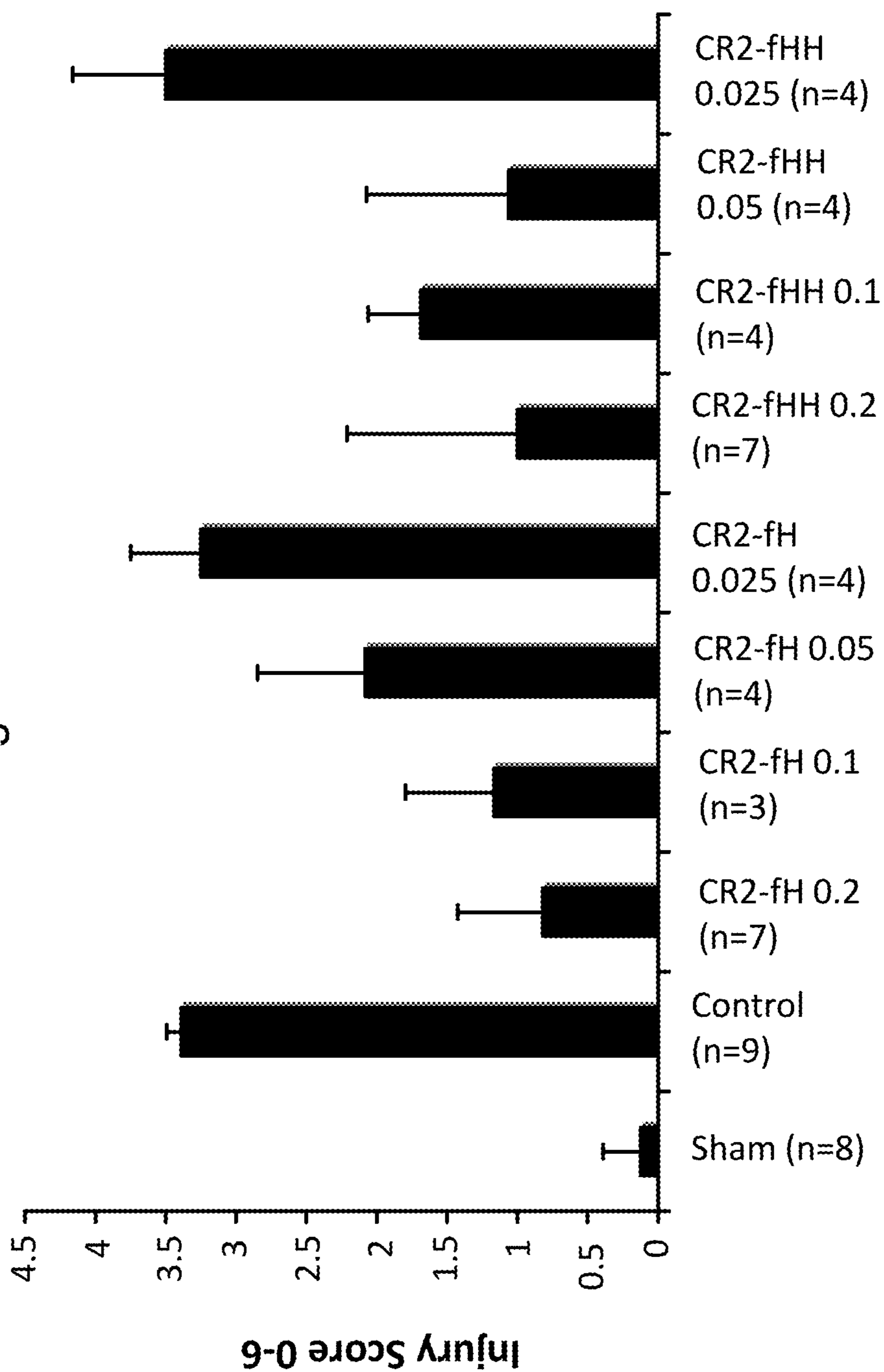


Figure 15B

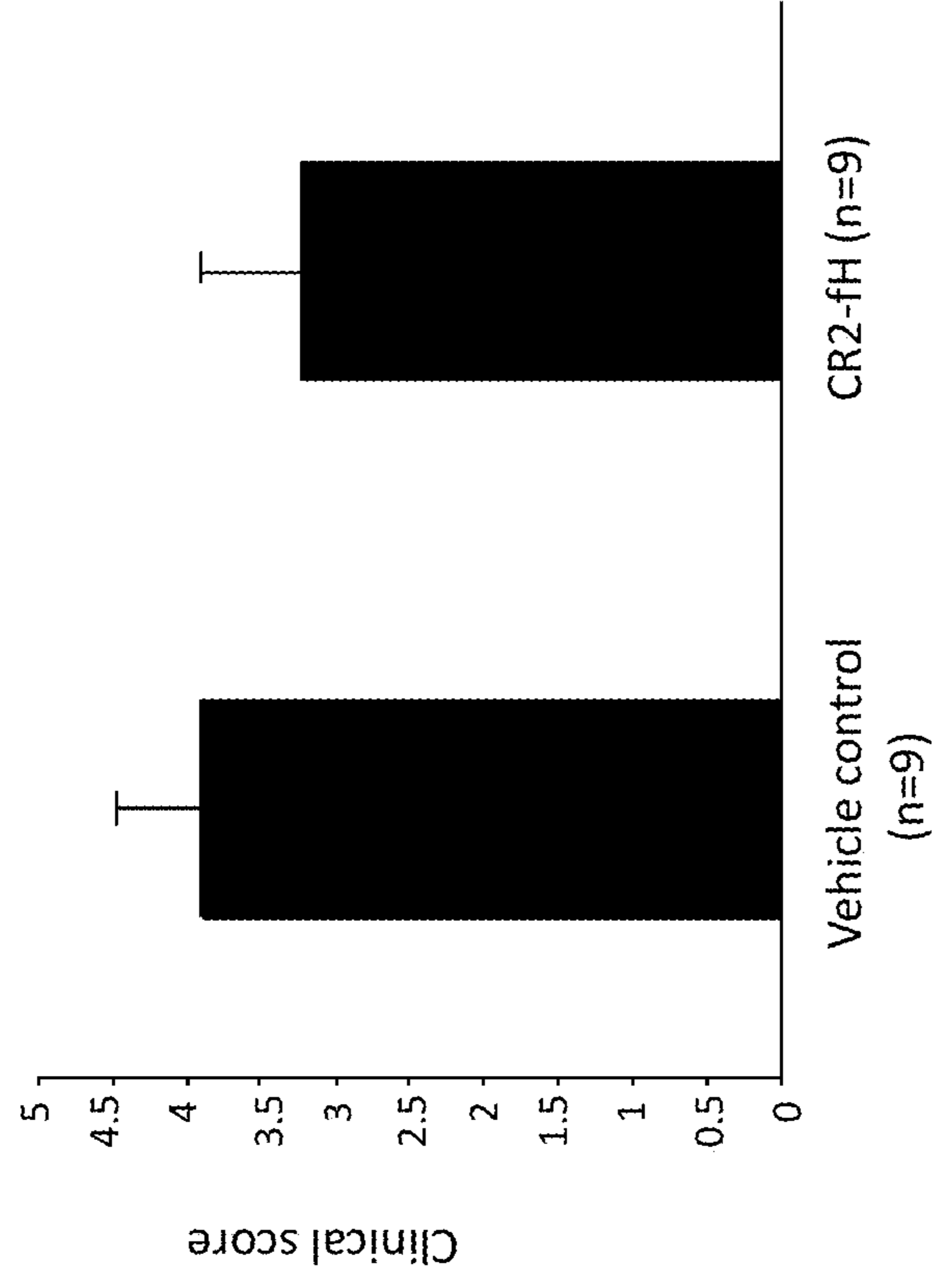


Figure 15A

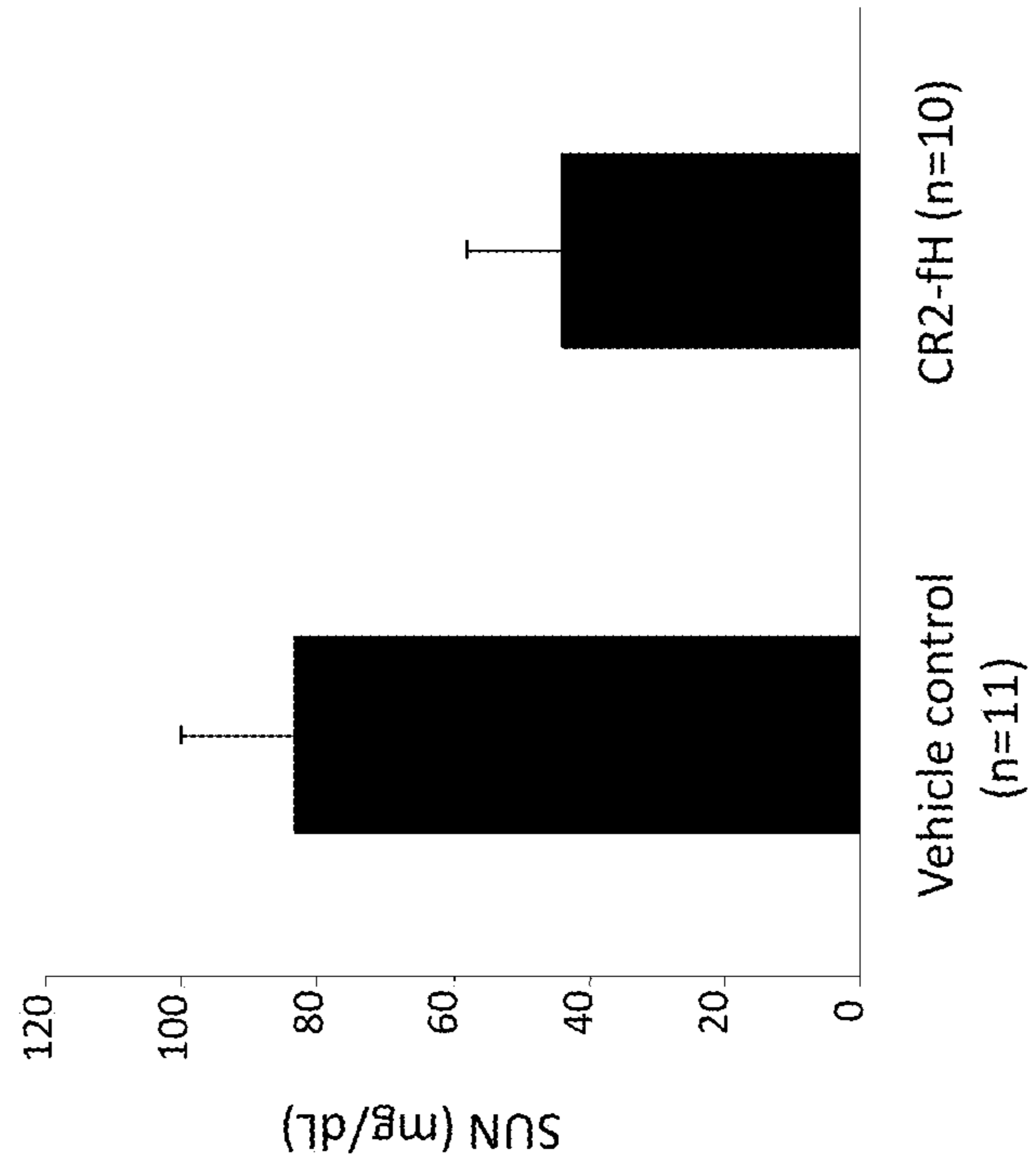


Figure 15D

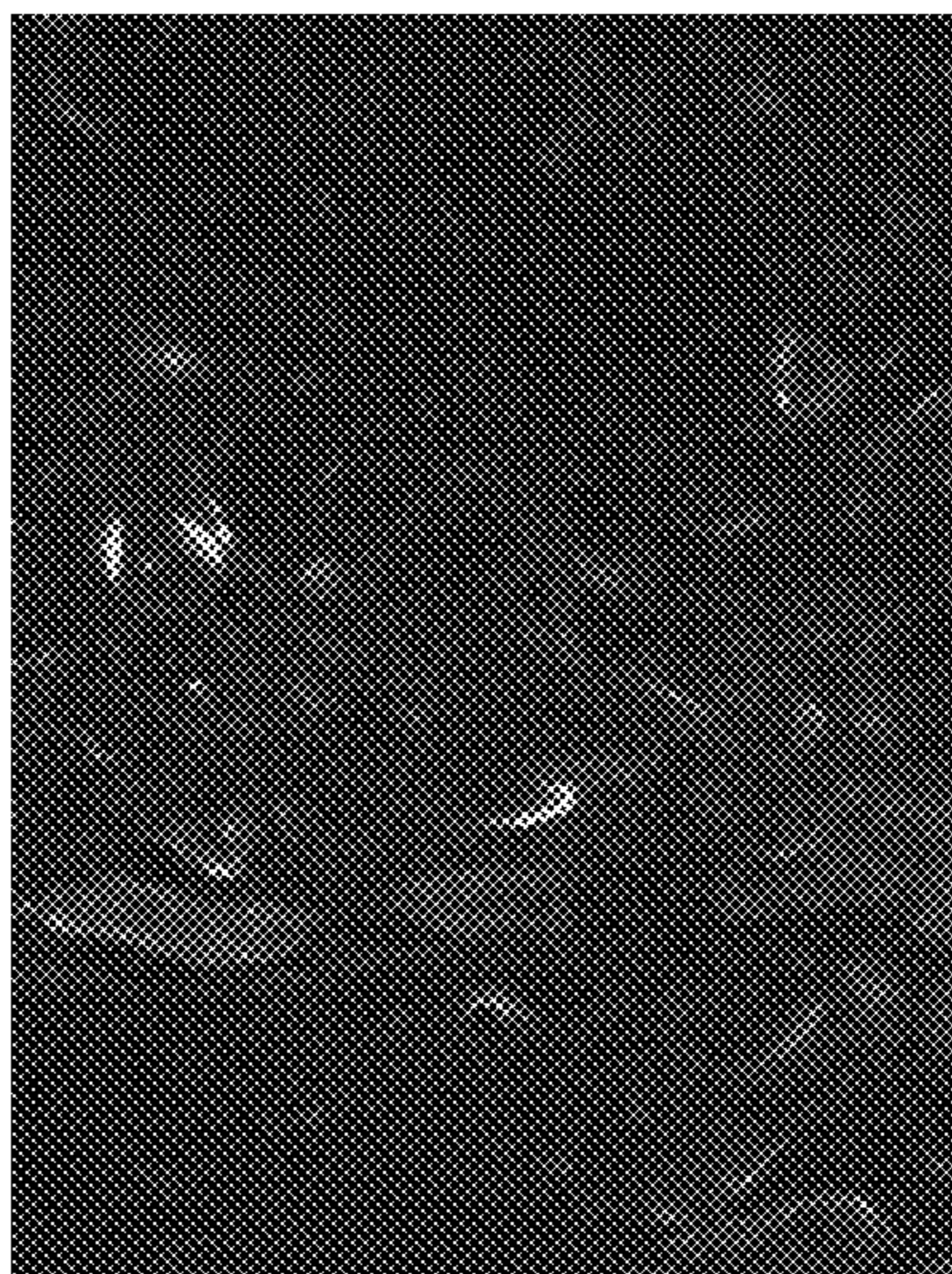


Figure 15C

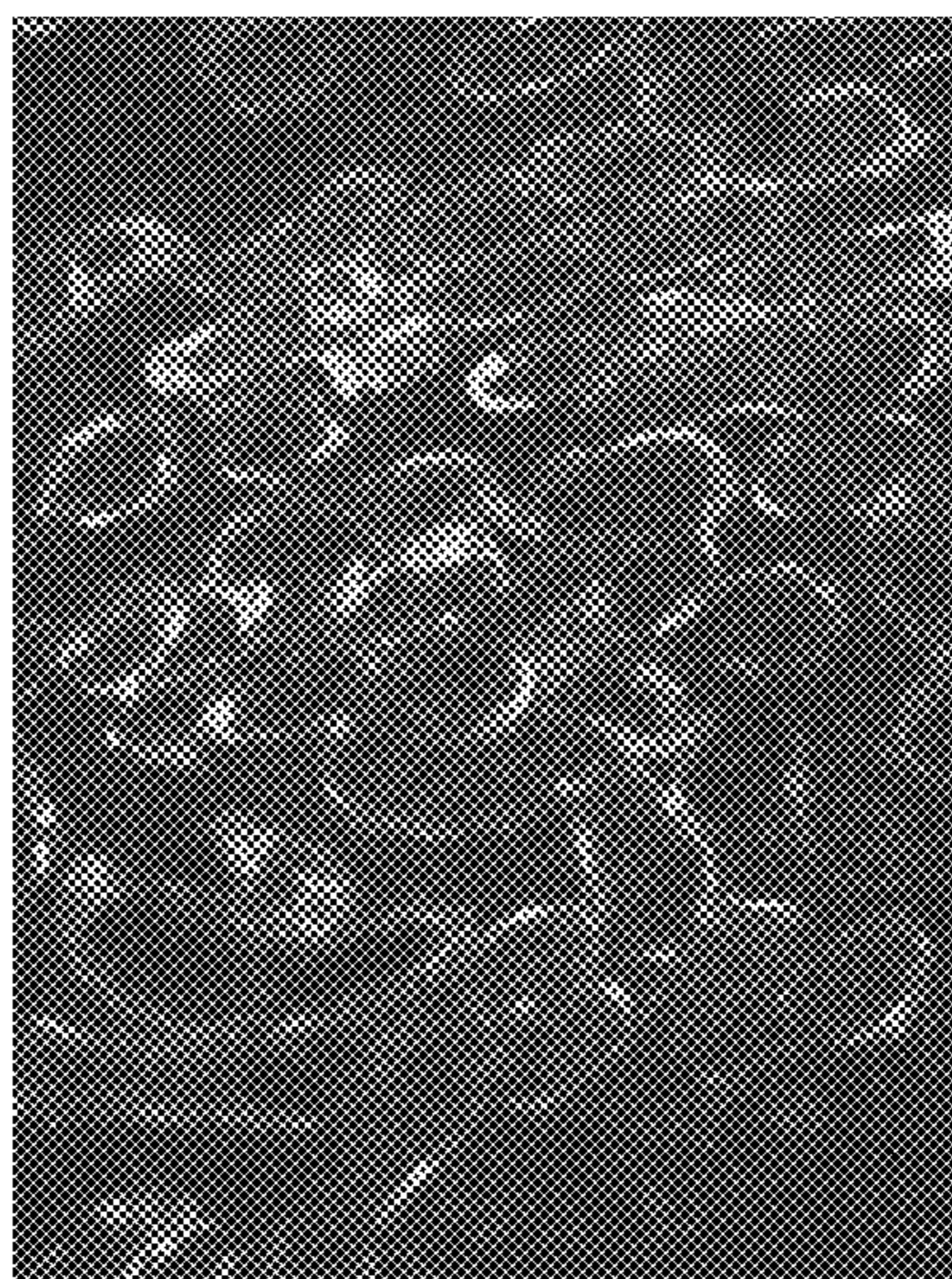


Figure 16

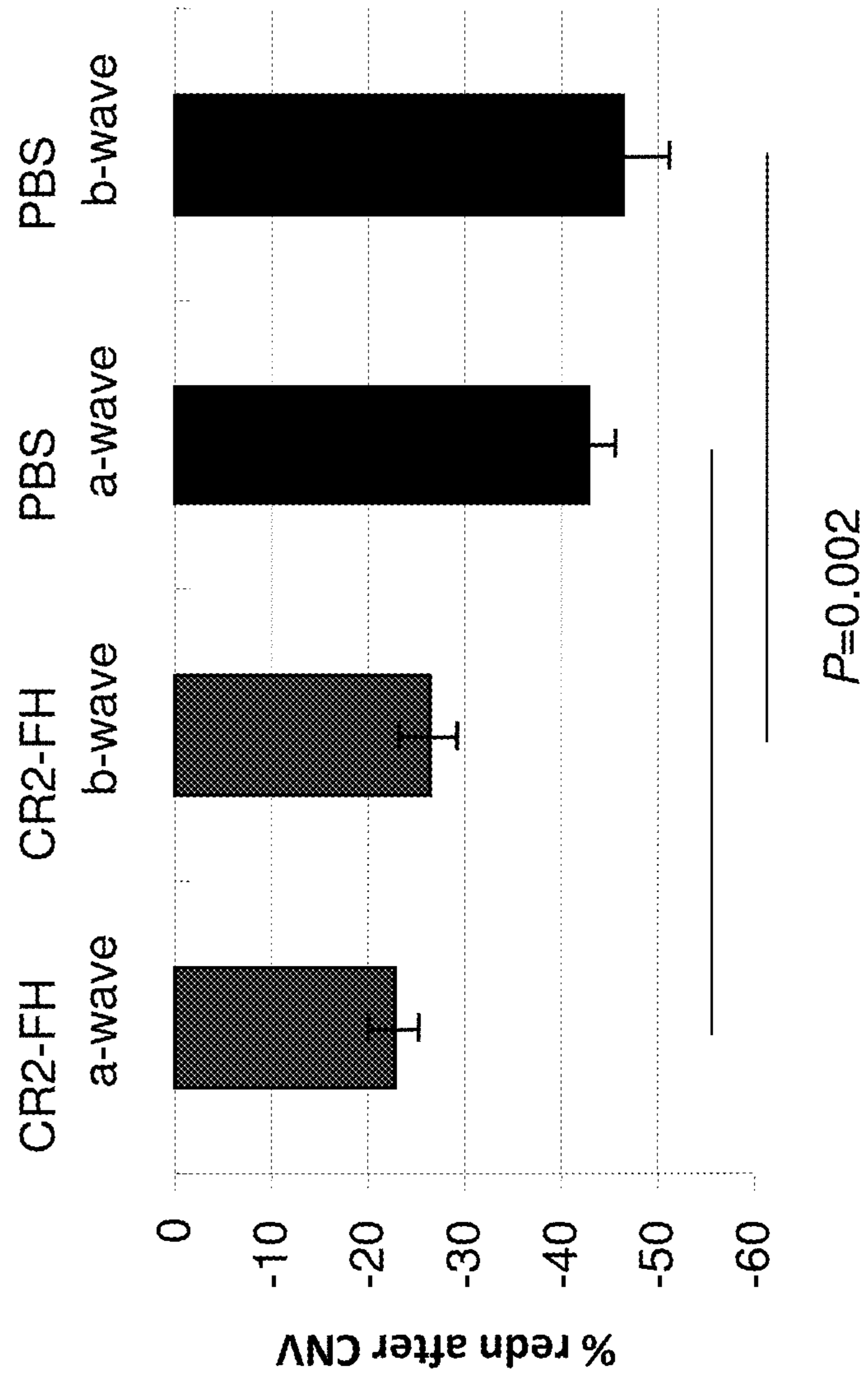


Figure 17B

CR2-FH

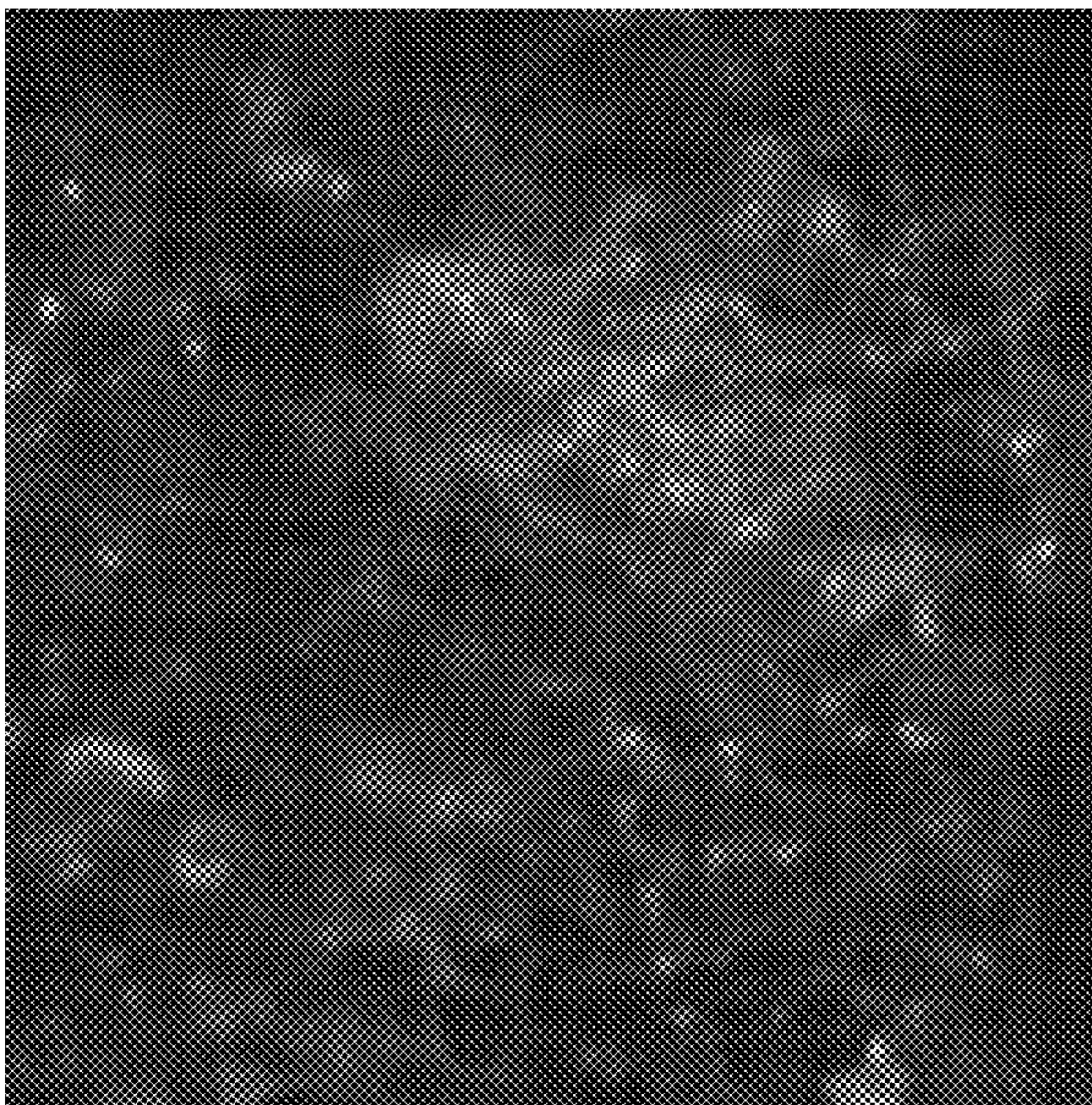


Figure 17A

PBS

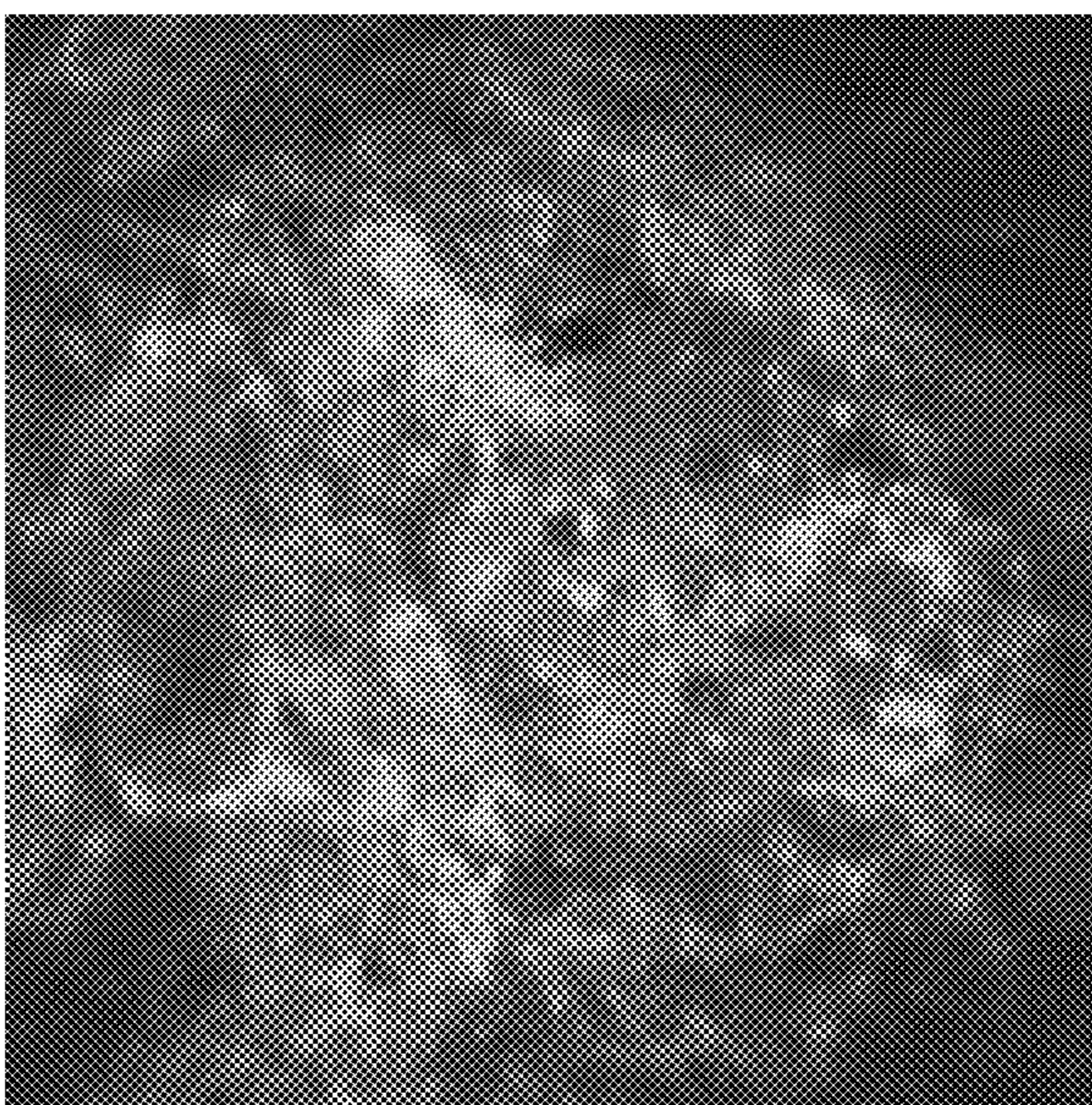


Figure 17C

$P=0.00$

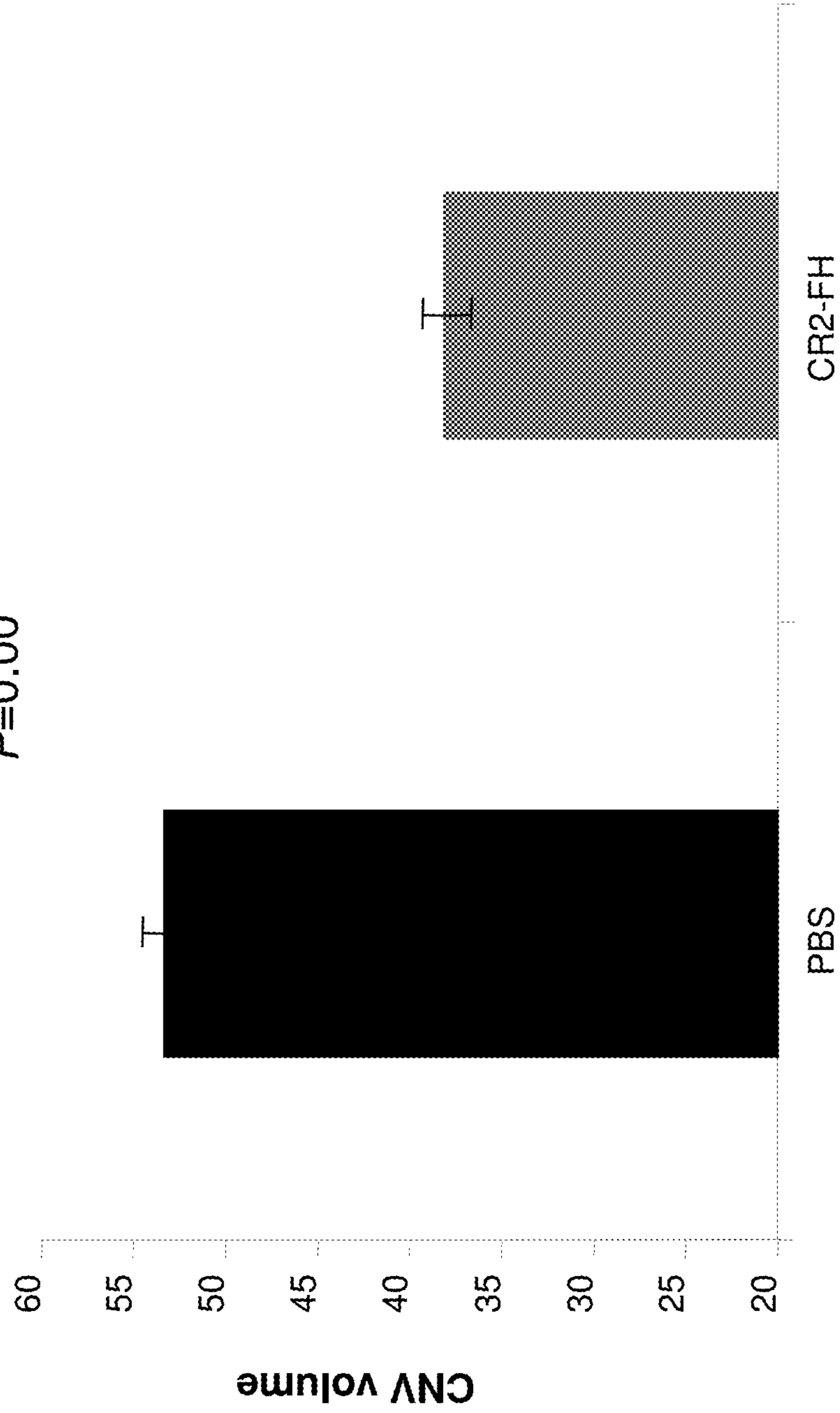


Figure 18B

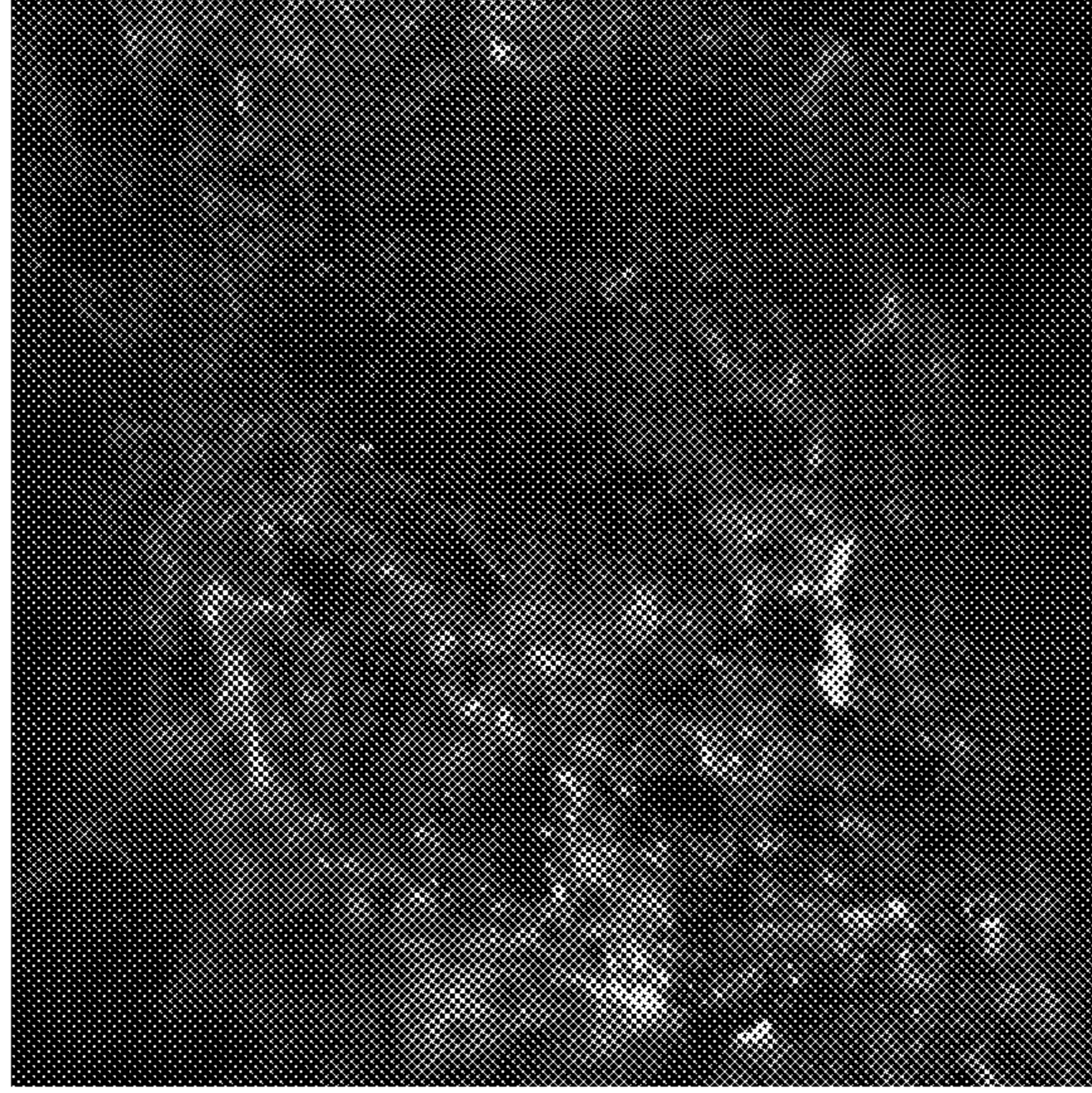


Figure 18A

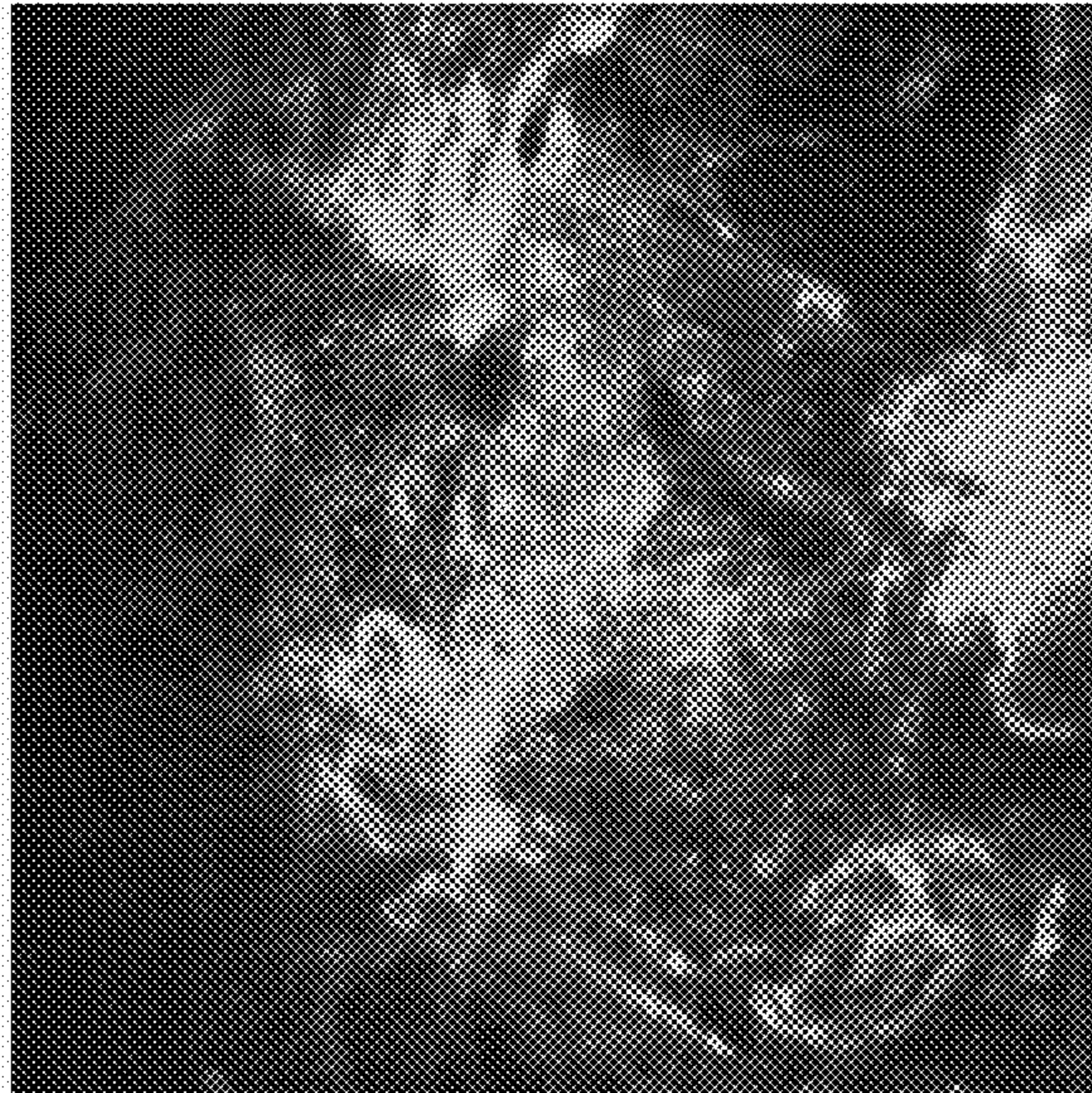


Figure 18C

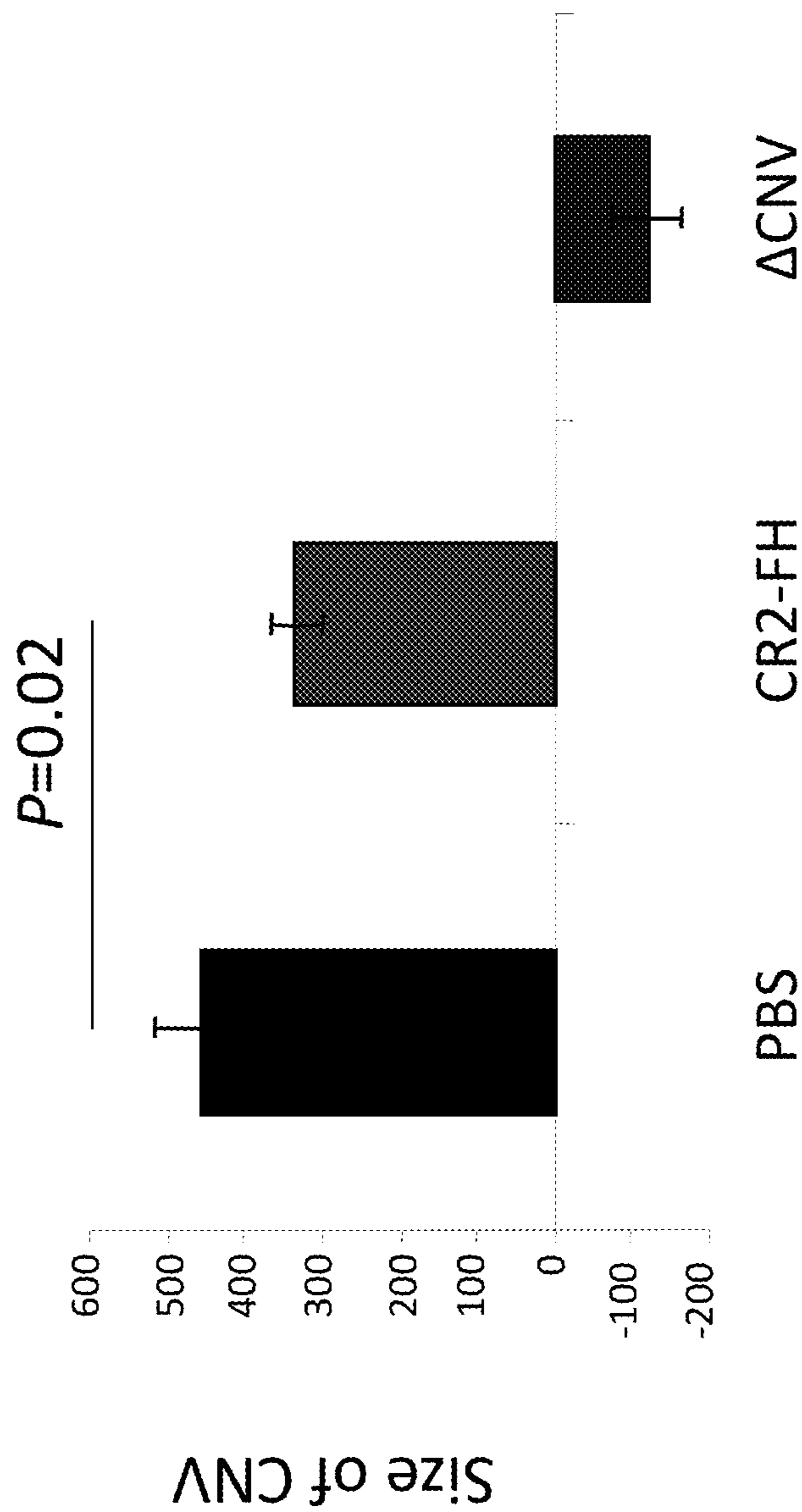


Figure 19

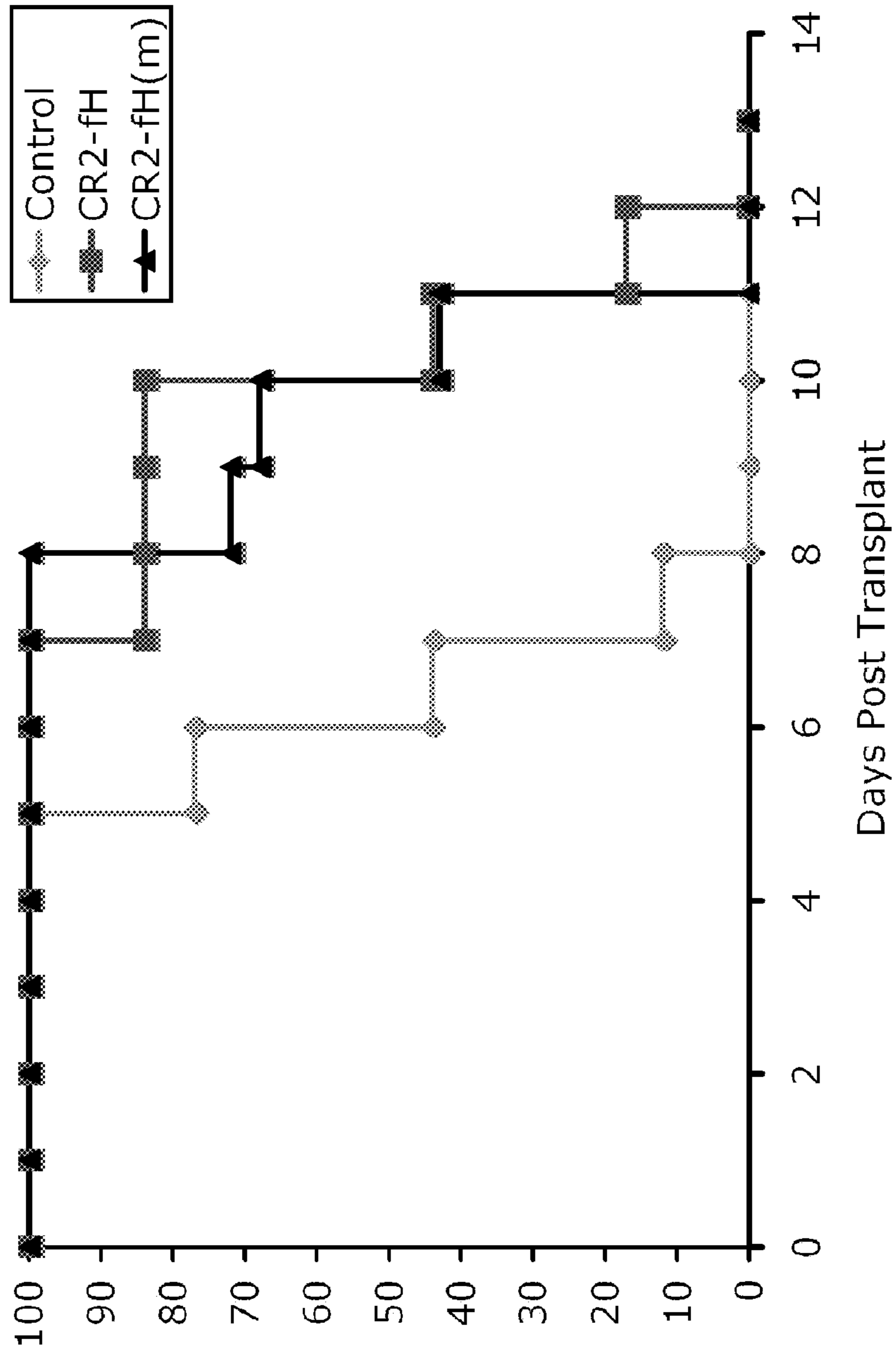


Figure 20

SEQ ID NO:21. human CR2-FH amino acid sequence

ISCGSPPPILNGRISYYSTPIAVGTVIRYSCSGTFRLLIGEKSLLCITKDKVDGTWDPAP
KCEYFNKYSSCPEPIVPGGYKIRGSTPYRHGDSVTFACKTNFSMNGNKSVMWCQANN
MWGPTRLPTCVSVFPLECPALPMIHNGHHTSENVGSIAPGLSVTYSCESGYLLVGEKII
NCLSSGKWSAVPPTCEEARKSLGRFPNGKVKEPPILRVGVTANFFCDEGYRLQGPPS
SRCVIAGQGVAVTKMPVCEEIFEDCNELPPRRNTEILTGSWSDQTYPEGTQAIYKCRP
GYRSLGNVIMVCRKGEWVALNPLRKCQKRPCGHPGDTPFGTFTLTGGNVFEYGVKA
VYTCNEGYQLLGEINYRECDTDGWTNDIPICEVVKCLPVTAPENKIVSSAMEPDRE
YHFGQAVRFVCNSGYKIEGDEEMHCSDDGFWSKEKPKCWEISCKSPDVINGSPISQKII
YKENERFQYKCNMGYEYSERGDAVCTESGWRPLPSCEEKSCDNPYIPNGDYSPLRIK
HRTGDEITYQCRNGFYPA TRGNTAKCTSTGWIPAPRCTLK

SEQ ID NO:22. human CR2-FH DNA sequence (including signal peptide)

GCCGC_{ca}CCATGGGAGCCGCTGGTCTGCTCGGCGTGTTCCTCGCCTTGGTGGCACC
TGGCGTCTGGGCATCAGCTGCGGTTCCCTCCACCAATCCTGAATGGCAGAATC
TCCTATTACTCCACACCAATCGCCGTCGGCACTGTGATCAGATACAGCTGTTCAG
GGACTTTTCGGCTGATCGGCGAGAAAAGCCTCCTCTGCATTACCAAGGATAAGGT
CGATGGGACATGGGATAAACCAGCTCCTAAGTGCGAGTACTTCAATAAGTATAGT
TCATGTCCAGAGCCCATTGTTCCCTGGTGGCTACAAGATTTCGGGGGAGCACACCCT
ATCGCCACGGTGACTCAGTGACCTTTGCTTGTA AAAACCAACTTCTCAATGAACGG
TAATAAGTCAGTGTGGTGT CAGGCCAATAATATGTGGGGTCCACACGACTCCCC
ACCTGTGTGTCCGTGTTCCCTTGGAATGCCCGCCCTGCCCATGATCCATAATGG
ACACCACACCAGCGAGAATGT CGGGAGTATCGCACCTGGATTGAGTGT CACCTAC
TCATGCGAGTCTGGCTACCTGCTTG TAGGTGAAAAAATTATTAATTGCTTGTCCTC
CGGCAAATGGAGTGCCGTTCCCCCAACTTGTGAAGAGGCCCGGTGCAAATCCCTC
GGCCGCTTCCCTAATGGTAAAGTTAAAGAGCCTCCAATCCTCAGAGTGGGGGTGA
CCGCTAACTTCTTCTGTGATGAAGGCTACCGGTTGCAGGGACCACCCAGTAGCCG
GTGTGTCATAGCTGGGCAGGGAGTGGCTTGGACAAAGATGCCCGTTTGTGAGGA
AATCTTCGAAGACTGTAATGAGCTGCCCCCAAGACGGAATACAGAGATCCTCAC
AGGCTCTTGGTCCGATCAA ACTTATCCAGAGGGTACCCAGGCAATTTACAAGTGC
AGACCTGGATACAGGAGCCTGGGCAATGTGATTATGGTGTGCCGCAAGGGGGAG
TGGGTGGCCCTTAATCCTCTCCGGAAGTGT CAGAAAAGACCATGCGGACACCCTG
GAGATACACCTTTCGGTACCTTTACCCTTACCGGCGGCAATGTCTTCGAGTATGG
CGTCAAGGCCGTGTACACTTGTAAACGAGGGATAACCAGCTGCTGGGGGAAATAAA
CTATCGTGAGTGTGACACTGACGGGTGGACTAACGACATCCCCATTTGCGAGGTG
GTCAAGTGCCCTTCTGTAAACCGCTCCCGAAAATGGTAAGATCGTATCTTCCGCAA
TGGAGCCTG_aTCGGGAATAC_{ca}CTTTGGACAAGCCGTTCCGGTTCGTATGTAATTCA
GGGTATAAAATTGAGGGCGATGAGGAGATGCACTGCAGTGTGACGGCTTTTGG
TCAAAGGAAAAGCCAAAGTGCGTAGAGATCAGTTGTAAGTCTCCTGACGTTATTA
ACGGGAGTCCCATCAGTCAGAAGATCATTTACAAGGAAAACGAGAGGTTCCAGT
ATAAATGCAATATGGGATATGAGTACTCCGAAAGAGGGGACGCCGTGTGCACAG
AGTCCGGATGGCGACCTTTGCCATCTTGTGAAGAAAAGTCTTGTGACAACCCCTA
TATTCCTAACGGAGATTACTCTCCTCTGCGCATCAAGCACCGAACTGGGGACGAG
ATCACTTACCAATGTTCGAAACGGCTTCTACCCTGCTACCAGAGGTAACACTGCCA
AGTGTACCAGCACCGGTTGGATTCCCGCCCCCAGATGCACACTTAAATGATAA

Figure 21

SEQ ID NO: 23. human CR2-FH2 amino acid sequence

ISCGSPPPILNGRISYYSTPIAVGTVIRYSCSGTFRLLIGEKSLLCITKDKVDGTWDPKAP
KCEYFNKYSSCPEPIVPGGYKIRGSTPYRHGDSVTFACKTNFSMNGNKS VWCQANN
MWGPTRLPTCVSVFPLECPALPMIHNGHHTSENVGSIAPGLSVTYSCESGYLLVGEKII
NCLSSGKWSA VPPTCEEARCKSLGRFPNGKVKEPPILRVGVTANFFCDEGYRLQGPPS
SRCVIAGQGVAVTKMPVCEEIFEDCNELPPRRNTEILTGSWSDQTYPEGTQAIYKCRP
GYRSLGNVIMVCRKGEWVALNPLRKCQKRPCGHPGDTFPGTFTLTGGNVFEYGVKA
VYTCNEGYQLLGEINYRECDTDGWTNDIPICEVVKCLPVTAPENKIVSSAMEPDRE
YHFGQAVRFVCNSGYKIEGDEEMHCSDDGFWSKEKPKCVEISCKSPDVINGSPISQKII
YKENERFQYKCNMGYEYSERGDVCTESGWRPLPSCEEKSCDNPYIPNGDYSPLRIK
HRTGDEITYQCRNGFY PATRGNTAKCTSTGWIPAPRCTEDCNELPPRRNTEILTGSWS
DQTYPEGTQAIYKCRPGYRSLGNVIMVCRKGEWVALNPLRKCQKRPCGHPGDTFPG
TFTLTGGNVFEYGVKAVYTCNEGYQLLGEINYRECDTDGWTNDIPICEVVKCLPVT
APENKIVSSAMEPDREYHFGQAVRFVCNSGYKIEGDEEMHCSDDGFWSKEKPKCVEI
SCKSPDVINGSPISQKIIYKENERFQYKCNMGYEYSERGDVCTESGWRPLPSCEEK
CDNPYIPNGDYSPLRIKHRTGDEITYQCRNGFY PATRGNTAKCTSTGWIPAPRCTLK

SEQ ID NO:24. human CR2-FH2 DNA sequence (including signal peptide)

CGCCGCCACCATGGGCGCAGCAGGCTTGTTGGGCGTGTTCCTGGCATTGGTGGCA
CCCGGCGTATTGGGCATTTTCATGCGGCTCTCCTCCACCCATTCTCAATGGAAGGA
TCTCCTACTACAGCACCCCATAGCTGTTCGGCACCGTTATCCGATACAGTTGTTCC
GGTACTTTCCGGCTTATCGGGCGAAAAGTCTTTGCTGTGCATTACCAAGGATAAAG
TGGACGGGACTTGGGACAAACCCGCACCTAAGTGCAGATATTTTAAACAAATATA
GCAGCTGCCCTGAGCCTATAGTACCCGGGGGTATAAAATCCGGGGCTCTACTCC
CTATCGTCATGGCGATTCTGTGACCTTCGCATGTAAAATAATTTTTCAATGAATG
GCAACAAGTCTGTATGGTGTCAAGCAAATAACATGTGGGGACCTACCCGCCTGCC
AACCTGTGTGTCAGTGTTTCCCCTGGAATGTCCAGCCCTCCCTATGATCCACAACG
GACATCACACCAGCGAAAACGTTGGATCCATCGCACCCAGGGCTCTCTGTGACTTA
CTCTTGCGAGTCCGGGTACCTGCTCGTGGGTGAAAAGATCATCAACTGCCTCAGT
AGTGGTAAATGGTCCGCGCTGCCTCCACATGTGAAGAGGCCCGGTGCAAGAGC
CTGGGCCCGGTTCCCCAACGGAAAAGTGAAGGAACCTCCTATCTTGAGGGTTGGTG
TGACCGCTAACTTTTTCTGCGACGAGGGGTACAGGCTCCAAGGGCCTCCCTCTAG
TCGGTGCATAATCGCCGGTCAAGGAGTCGCATGGACTAAGATGCCTGTGTGTGAG
GAGATTTTCGAGGATTGTAATGAATTGCCACCCAGGAGAAATACTGAAATCCTGA
CAGGCTCTTGGTCTGATCAGACTTATCCAGAAGGCACCCAGGCCATTTACAAGTG
TCGGCCTGGATACAGATCTCTGGGAAATGTGATCATGGTATGTAGGAAAGGAGA
GTGGGTGGCTTTGAACCCCTCCGCAAGTGTGAGAAAAGACCATGCGGGCATCCT
GGAGACACCCCATTCGGGACATTTACACTGACAGGCGGAAACGTATTTGAGTAC
GGAGTCAAGGCCGTTTATACATGTAACGAAGGGTATCAACTGCTGGGAGAAATC
AACTATAGGGAGTGCACACTGACGGATGGACAAACGACATTCCAATCTGCGAA
GTGGTGAAATGTCTTCCAGTTACAGCCCCTGAAAACGGGAAAATCGTGTCTCCG
CTATGGAGCCTGACCGGGAATATCATTTCCGGCCAGGCCGTTAGATTCGTGTGTAA
TAGCGGCTACAAAATCGAGGGCGACGAAGAAATGCATTGCAGCGATGACGGGTT
CTGGAGCAAGGAGAAGCCTAAATGCGTTCGAAATTTTCATGCAAGAGTCCCGACGT
CATAAACGGTTCTCCAATTTCCAGAAAGATCATTTATAAGGAGAATGAGCGGTT
CAGTATAAGTGTAATATGGGCTACGAGTACAGCGAACGCGGTGACGCCGTGTGT
ACCGAAAGTGGCTGGAGACCACTGCCTAGTTGCGAGGAGAAATCCTGCGACAAC
CCTTATATTCCCAACGGGGACTACTCTCCTCTGAGAATCAAGCATCGGACTGGCG
ACGAGATTACTTACCAATGCAGGAACGGATTCTATCCAGCAACTCGGGGCAATAC

Figure 21 (Cont.)

CGCTAAGTGTACCTCCACAGGCTGGATACCCGCTCCTAGATGTACAGAGGACTGC
AATGAACTGCCACCTCGGCGCAATACAGAAATTTTGACTGGATCATGGTCTGACC
AGACTTACCCCGAGGGCACCCAGGCCATCTACAAATGTAGGCCCGGTTATCGAA
GTTTGGGTAAACGTGATTATGGTGTGTCGAAAAGGTGAATGGGTAGCACTCAATCC
CCTCCGTAAATGCCAGAAGCGTCCTTGTGGGCACCCAGGCGATAACCCCTTTTGG
ACTTTCACCCTGACTGGAGGAAACGTCTTTGAATATGGTGTGAAAGCCGTGTACA
CATGCAATGAAGGGTACCAACTGCTCGGAGAGATAAACTATCGGGAGTGCGATA
CAGATGGATGGACCAATGATATAACCAATCTGCGAGGTGGTGAAGTGTCTCCAGT
CACCGCTCCTGAGAACGGAAAGATCGTCAGTTCTGCTATGGAACCTGACAGGGA
ATACCACTTTGGGCAAGCCGTCCGCTTCGTGTGCAATTCAGGGTACAAGATAGAA
GGCGACGAAGAGATGCACTGTTCCGACGATGGTTTCTGGTCTAAGGAGAAGCCT
AAATGTGTCGAGATTAGCTGCAAGTCTCCCGATGTTATTAACGGCTCTCCCATCTC
TCAAAAAATTATTTATAAGGAAAACGAAAGATTTTCAGTACAAGTGCAATATGGG
TTATGAGTACAGTGAACGTGGAGACGCCGTGTGCACAGAGTCCGGGTGGCGTCC
ACTGCCAGCTGCGAAGAAAAATCCTGTGACAACCCCTACATCCCAATGGCGAC
TATCCCCCTGCGCATCAAACATCGTACTGGCGATGAAATTACTTACCAGTGCC
GCAACGGGTCTACCCTGCCACCCGGGGTAACACAGCCAAATGCACCTCCACCGG
ATGGATCCCCGCCCCACGCTGTACCTTGAAATGATGA

Figure 22A

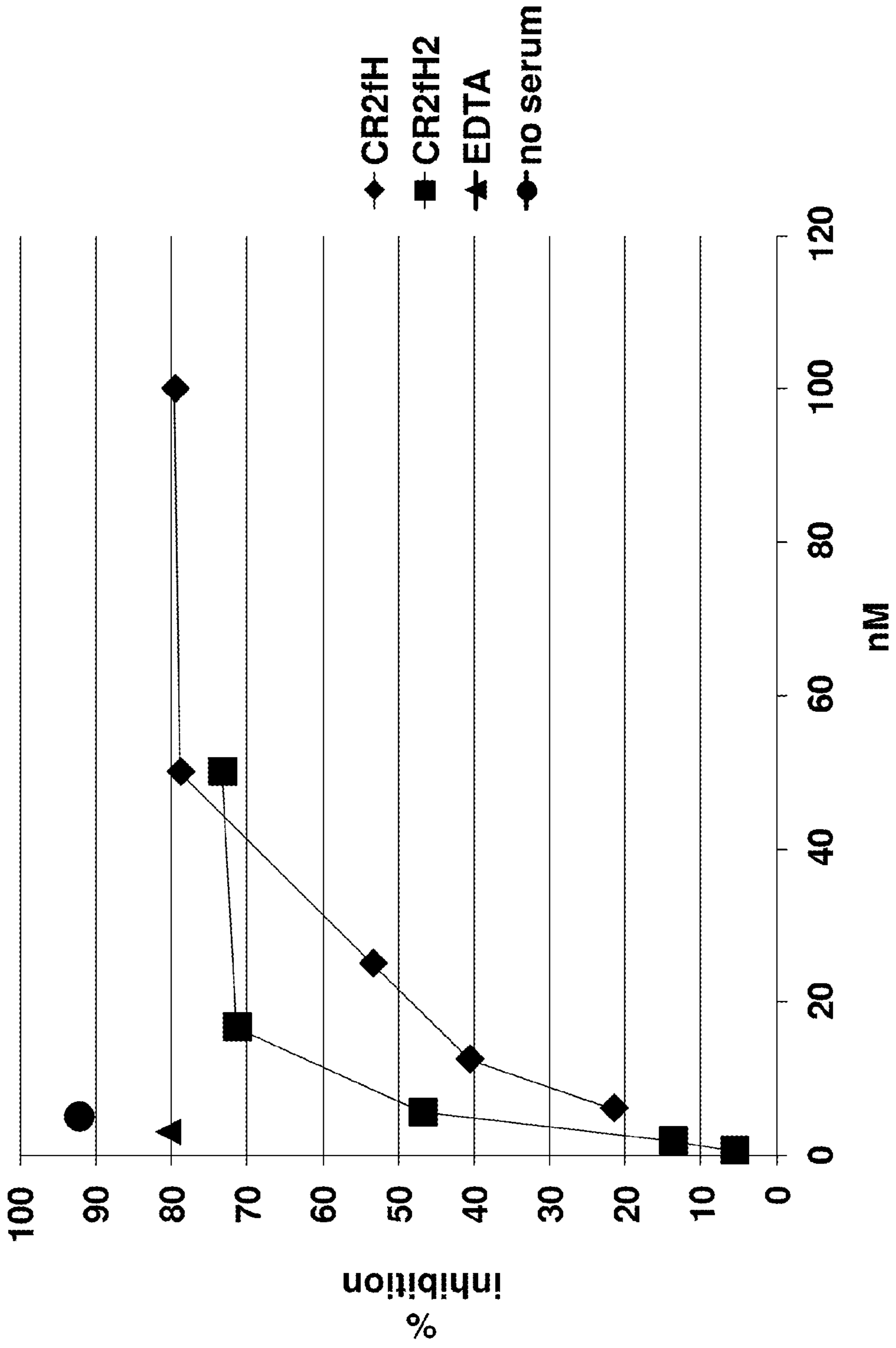


Figure 22B

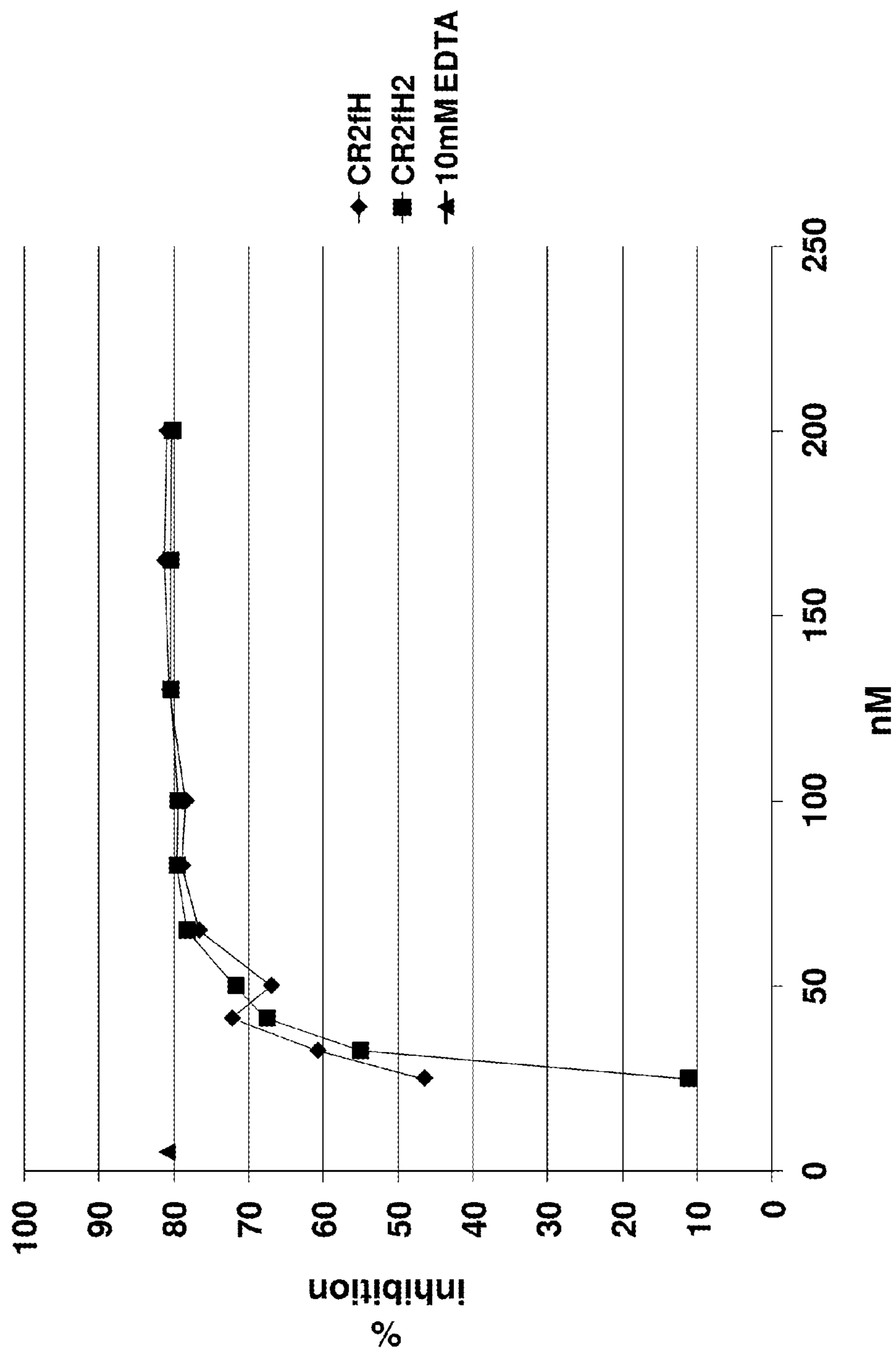


Figure 23

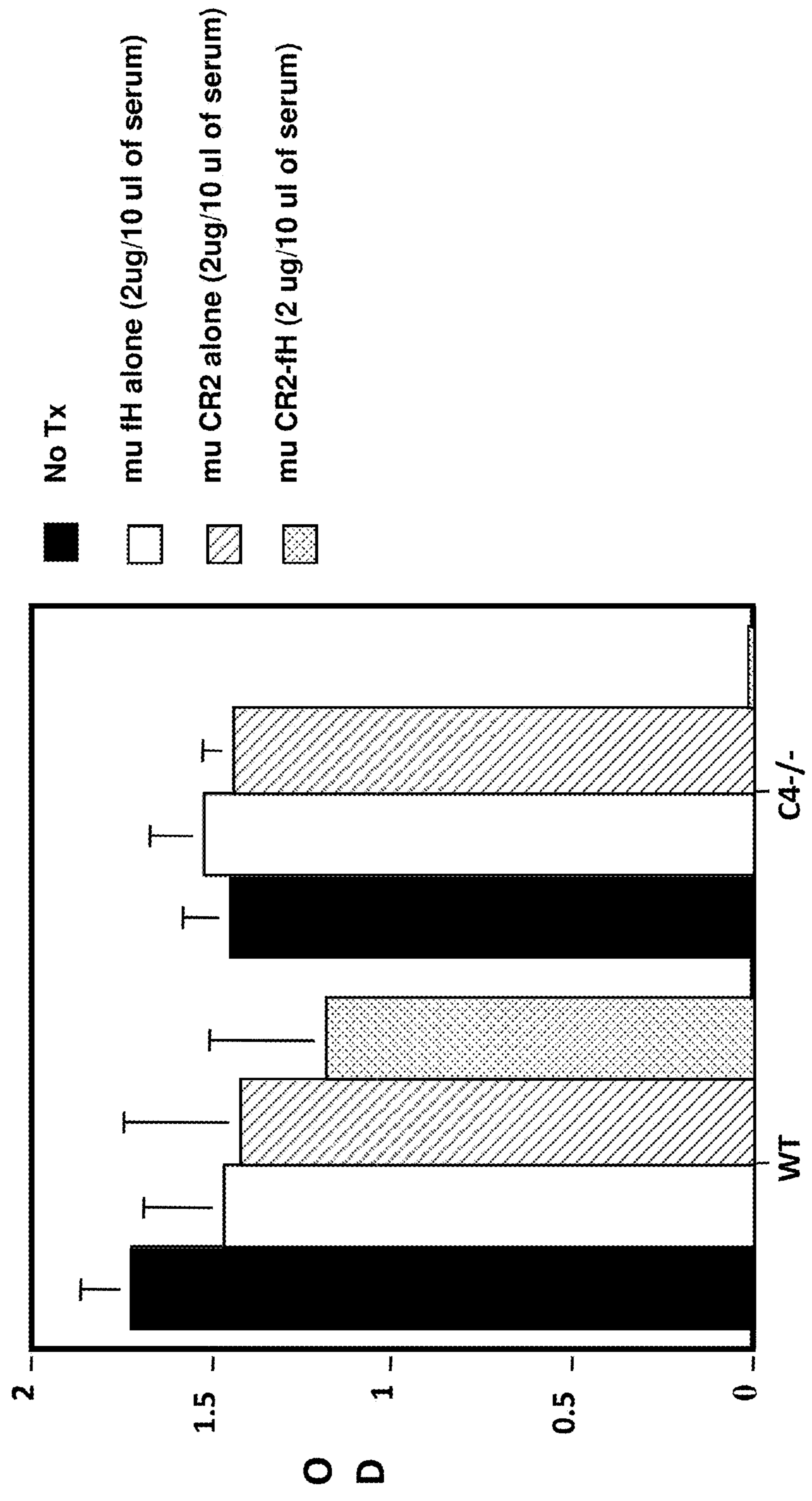
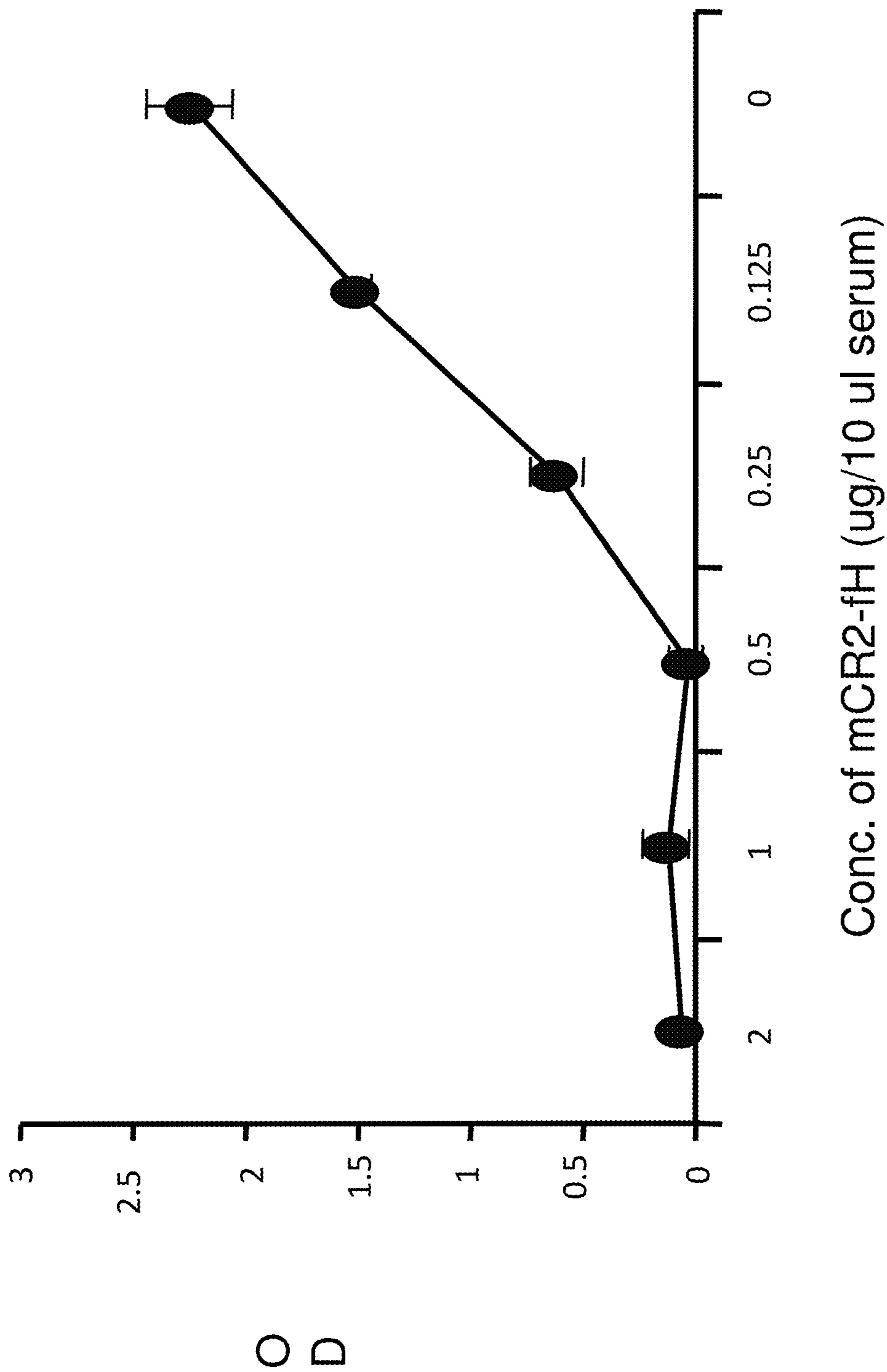


Figure 24



TARGETING COMPLEMENT FACTOR H FOR TREATMENT OF DISEASES

RELATED APPLICATIONS

This application is a continuation of U.S. application Ser. No. 12/782,526, filed on May 18, 2010, which is a continuation of U.S. application Ser. No. 11/821,370, filed on Jun. 21, 2007, which claims benefit of U.S. provisional application No. 60/815,748, filed on Jun. 21, 2006.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with Government support under Grant (Contract) Nos.: AI47469, AI31105, and EY13520 awarded by the National Institutes of Health.

TECHNICAL FIELD

This application pertains to compositions and methods of treating diseases in which the alternative complement pathway is implicated. Specifically, the application pertains to a CR2-FH molecule and uses thereof for treating diseases in which the alternative complement pathway is implicated.

BACKGROUND

Complement is the collective term for a series of blood proteins and is a major effector mechanism of the immune system. Complement plays an important role in the pathology of many autoimmune, inflammatory, and ischemic diseases, and is also responsible for many disease states associated with bioincompatibility. Inappropriate complement activation and its deposition on host cells can lead to complement-mediated cell lysis of target structures, as well as tissue destruction due to the generation of powerful mediators of inflammation.

Complement can be activated by one of the three pathways, the classical, lectin, and alternative pathways. The classical pathway is activated through the binding of the complement system protein C1q to antigen-antibody complexes, pentraxins, or apoptotic cells. The pentraxins include C-reactive protein and serum amyloid P component. The lectin pathway is initiated by microbial saccharides via the mannose-binding lectin. The alternative pathway is activated on surfaces of pathogens that have neutral or positive charge characteristics and do not express or contain complement inhibitors. This is due to the process termed "tickover" of C3 that occurs spontaneously, involving the interaction of conformationally altered C3 with factor B, and results in the fixation of active C3b on pathogens or other surfaces. The alternative pathway can also be initiated when certain antibodies block endogenous regulatory mechanisms, by IgA-containing immune complexes, or when expression of complement regulatory proteins is decreased. In addition, the alternative pathway is activated by a mechanism called the "amplification loop" when C3b that is deposited onto targets via the classical or lectin pathway then binds factor B. Muller-Eberhard, 1988, *Ann. Rev. Biochem.* 57:321. For example, Holers and collaborators have shown that the alternative pathway is amplified at sites of local injury when inflammatory cells are recruited following initial complement activation. Girardi et al., *J. Clin. Invest.* 2003, 112:1644. Dramatic complement amplification through the alternative pathway then occurs through a mechanism that involves either the additional generation of injured cells that fix complement, local synthesis of alternative pathway components, or more likely because these infiltrating

inflammatory cells that carry preformed C3 and properdin greatly increase activation specifically at that site.

Alternative pathway activation is initiated when circulating factor B binds to activated C3. This complex is then cleaved by circulating factor D to yield an enzymatically active fragment, C3bBb. C3bBb cleaves C3 generating C3b, which drives inflammation and also further amplifies the activation process, generating a positive feedback loop. Factor H (FH) is a key regulator (inhibitor) of the alternative complement pathway. It functions by competing with factor B for binding to C3b. Binding of C3b to Factor H also leads to degradation of C3b by factor I to the inactive form C3bi (also designated iC3b), thus exerting a further check on complement activation. The actual plasma concentration of factor H is approximately 500 µg/ml, providing complement regulation in the fluid phase, but its binding to cells is a regulated phenomenon that is enhanced by the presence of a negatively charged surface as well as fixed C3b, C3bi, or C3d. Jozsi et al., *Histopathol* (2004) 19:251-258.

The down-regulation of complement activation has been demonstrated to be effective in treating several disease indications in animal models and in ex vivo studies, e.g. systemic lupus erythematosus and glomerulonephritis (Y. Wang et al., *Proc. Natl. Acad. Sci.*; 1996, 93: 8563-8568), rheumatoid arthritis (Y. Wang et al., *Proc. Natl. Acad. Sci.*, 1995; 92: 8955-8959), cardiopulmonary bypass and hemodialysis (C. S. Rinder, *J. Clin. Invest.*, 1995; 96: 1564-1572), hyperacute rejection in organ transplantation (T. J. Kroshus et al., *Transplantation*, 1995; 60: 1194-1202), myocardial infarction (J. W. Homeister et al., *J. Immunol.*, 1993; 150: 1055-1064; H. F. Weisman et al., *Science*, 1990; 249: 146-151), reperfusion injury (E. A. Amsterdam et al., *Am. J. Physiol.*, 1995; 268: H448-H457), and adult respiratory distress syndrome (R. Rabinovici et al., *J. Immunol.*, 1992; 149: 1744-1750). In addition, other inflammatory conditions and autoimmune/immune complex diseases are also closely associated with complement activation (B. P. Morgan. *Eur. J. Clin. Invest.*, 1994; 24: 219-228), including thermal injury, severe asthma, anaphylactic shock, bowel inflammation, urticaria, angioedema, vasculitis, multiple sclerosis, myasthenia gravis, membranoproliferative glomerulonephritis, and Sjogren's syndrome. Complement inhibitors and uses thereof are also disclosed in WO04/045520 and U.S. Pat. No. 6,521,450.

The disclosures of all publications, patents, patent applications and published patent applications referred to herein are hereby incorporated herein by reference in their entirety.

BRIEF SUMMARY OF THE INVENTION

The invention in one aspect provides a CR2-FH molecule comprising: a) a CR2 portion comprising a CR2 or a fragment thereof, and b) a FH portion comprising a FH or a fragment thereof. In some embodiments, there is provided a CR2-FH molecule comprising: a) a CR2 portion comprising a CR2 or a fragment thereof, and b) a FH portion comprising a FH or a fragment thereof, wherein the CR2-FH molecule is capable of binding to a CR2 ligand and wherein the CR2-FH molecule is capable of inhibiting complement activation of the alternative pathway. In some embodiments, there is provided an isolated CR2-FH molecule. In some embodiments, there is provided a composition (such as a pharmaceutical composition) comprising a CR2-FH molecule. In some embodiments, the CR2 portion and the FH portion are directly or indirectly fused to each other in the form of a fusion protein. In some embodiments, the CR2 portion and the FH portion are linked via a

chemical crosslinker. In some embodiments, the CR2 portion and the FH portion are non-covalently linked.

In some embodiments, there is provided a CR2-FH fusion protein comprising: a) a CR2 portion comprising a CR2 or a fragment thereof, and b) a FH portion comprising a FH or a fragment thereof. In some embodiments, there is provided a CR2-FH molecule comprising: a) a CR2 portion comprising a CR2 or a fragment thereof, and b) a FH portion comprising a FH or a fragment thereof, wherein the CR2-FH molecule is capable of binding to a CR2 ligand and wherein the CR2-FH molecule is capable of inhibiting complement activation of the alternative pathway. In some embodiments, the CR2 portion and the FH portion are directly fused (i.e., linked) to each other. In some embodiments, the CR2 portion and the FH portion are linked via an amino acid linker sequence. In some embodiments, the C-terminus of the CR2 portion is linked (directly or indirectly) to the N-terminus of the FH portion. In some embodiments, the N-terminus of the CR2 portion is linked (directly or indirectly) to the C-terminus of the FH portion.

In some embodiments, the CR2-FH molecule comprises two or more (such as any of two, three, four, five, or more) CR2 portions. These CR2 portions may be the same or different, for example in terms of amino acid sequences, structures, and/or functions. For example, in some embodiments, the CR2-FH molecule (such as a CR2-FH fusion protein) comprises: 1) two or more CR2 portions comprising a CR2 or a fragment thereof, and 2) an FH portion comprising a FH or a fragment thereof. In some embodiments, the CR2-FH molecule (such as a CR2-FH fusion protein) comprises: 1) two or more CR2 portions comprising a CR2 or a fragment thereof, and 2) an FH portion comprising a FH or a fragment thereof, wherein the CR2-FH molecule is capable of binding to a CR2 ligand and wherein the CR2-FH molecule is capable of inhibiting complement activation of the alternative pathway.

In some embodiments, the CR2-FH molecule comprises two or more (such as any of two, three, four, five, or more) FH portions. These FH portions may be the same or different, for example in terms of amino acid sequences, structures, and/or functions. For example, in some embodiments, the CR2-FH molecule (such as a CR2-FH fusion protein) comprises: 1) a CR2 portion comprising a CR2 or a fragment thereof, and 2) two or more FH portions comprising a FH or a fragment thereof. In some embodiments, the CR2-FH molecule (such as a CR2-FH fusion protein) comprises: 1) a CR2 portion comprising a CR2 or a fragment thereof, and 2) two or more (such as two) FH portions comprising a FH or a fragment thereof, wherein the CR2-FH molecule is capable of binding to a CR2 ligand and wherein the CR2-FH molecule is capable of inhibiting complement activation of the alternative pathway.

In some embodiments, the CR2-FH molecule (such as a CR2-FH fusion protein) comprises: 1) two or more CR2 portions comprising a CR2 or a fragment thereof, and 2) two or more FH portions comprising a FH or a fragment thereof. In some embodiments, the CR2-FH molecule (such as a CR2-FH fusion protein) comprises: 1) two or more CR2 portions comprising a CR2 or a fragment thereof, and 2) two or more (such as two) FH portions comprising a FH or a fragment thereof, wherein the CR2-FH molecule is capable of binding to a CR2 ligand and wherein the CR2-FH molecule is capable of inhibiting complement activation of the alternative pathway.

In some embodiments, the CR2-FH molecule (such as a CR2-FH fusion protein) comprises: 1) full length CR2; and 2) a FH portion comprising a FH or a fragment thereof. In some embodiments, the CR2-FH molecule (such as a CR2-FH

fusion protein) comprises: 1) a fragment of CR2, and 2) a FH portion comprising a FH or a fragment thereof. In some embodiments, the CR2-FH molecule (such as a CR2-FH fusion protein) comprises: 1) a CR2 portion comprising at least the first two N-terminal SCR domains of CR2, and b) a FH portion comprising a FH or a fragment thereof. In some embodiments, the CR2-FH molecule (such as a CR2-FH fusion protein) comprises: 1) a CR2 portion comprising at least the first four N-terminal SCR domains of CR2, and b) a FH portion comprising a FH or a fragment thereof. In some embodiments, the CR2-FH molecule is capable of binding to a CR2 ligand and inhibiting complement activation of the alternative pathway. In some embodiments, the CR2-FH molecule comprises two or more FH portions. In some embodiments, the FH portion comprises a full length FH. In some embodiments, the FH portion comprises a fragment of FH. In some embodiments, the FH portion comprises at least the first four N-terminal SCR domains of FH. In some embodiments, the FH portion comprises at least the first five N-terminal SCR domains of FH. In some embodiments, the FH portion lacks a heparin binding site. In some embodiments, the FH portion comprises a FH or a fragment thereof having a polymorphism that is protective against age-related macular degeneration.

In some embodiments, there is provided a CR2-FH molecule (such as a CR2-FH fusion protein) comprising: a) CR2 portion comprising a ligand binding site that is any of (and in some embodiments selected from the group consisting of) (1) a site on strand B and the B-C loop of CR2 SCR comprising the segment G98-G99-Y100-K101-I102-R103-G104-S105-T106-P107-Y108 with respect to SEQ ID NO: 1, (2) a site on the B strand of CR2 SCR2 comprising position K119 with respect to SEQ ID NO:1, (3) a segment comprising V149-F150-P151-L152 with respect to SEQ ID NO:1, and (4) a segment of CR2 SCR2 comprising T120-N121-F122 with respect to SEQ ID NO:1; and (b) a FH portion comprising a FH or a fragment thereof. In some embodiments, the CR2-FH molecule is capable of binding to a CR2 ligand and inhibiting complement activation of the alternative pathway. In some embodiments, the CR2 portion further comprises sequences required to maintain the three dimensional structure of the ligand binding site. In some embodiments, the CR2-FH molecule comprises two or more FH portions. In some embodiments, the FH portion comprises a full length FH. In some embodiments, the FH portion comprises a fragment of FH. In some embodiments, the FH portion comprises at least the first four N-terminal SCR domains of FH. In some embodiments, the FH portion comprises at least the first five N-terminal SCR domains of FH. In some embodiments, the FH portion lacks a heparin binding site. In some embodiments, the FH portion comprises a FH or a fragment thereof having a polymorphism that is protective against age-related macular degeneration.

In some embodiments, there is provided a CR2-FH molecule (such as a CR2-FH fusion protein) comprising: a) a CR2 portion comprising at least the first two N-terminal SCR domains of CR2, and b) a FH portion comprising at least the first four N-terminal SCR domains of FH. In some embodiments, the CR2-FH molecule is capable of binding to a CR2 ligand and inhibiting complement activation of the alternative pathway. In some embodiments, the CR2 portion comprises at least the first 3, 4, 5, 6, 7, or more N-terminal SCR domains of CR2. In some embodiments, the FH portion comprises at least the first 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or more N-terminal SCR domains of FH. In some embodiments, the CR2-FH molecule (such as a CR2-FH fusion protein) comprises (and in some embodiments consists of or consists essentially

of): a) a CR2 portion comprising the first four N-terminal SCR domains of CR2, and b) a FH portion comprising the first five N-terminal SCR domains of FH. In some embodiments, the CR2-FH molecule (such as a CR2-FH fusion protein) comprises (and in some embodiments consists of or consists essentially of): a) a CR2 portion comprising the first four N-terminal SCR domains of CR2, and b) two or more (such as two) FH portions comprising the first five N-terminal SCR domains of FH. In some embodiments, the CR2-FH molecule comprises (and in some embodiments consists of or consists essentially of): a) a CR2 portion comprising amino acids 23 to 271 of SEQ ID NO:1, and b) a FH portion comprising amino acids 21 to 320 of SEQ ID NO:2. In some embodiments, the CR2-FH molecule comprises (and in some embodiments consists of or consists essentially of): a) a CR2 portion comprising amino acids 23 to 271 of SEQ ID NO:1, and b) two or more (such as two) FH portions comprising amino acids 21 to 320 of SEQ ID NO:2.

In some embodiments, the CR2-FH is a fusion protein having an amino acid sequence of any of SEQ ID NO:3, SEQ ID NO:21, and SEQ ID NO:23. In some embodiments, the CR2-FH molecule is a fusion protein having amino acid sequence that is at least about any of 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to that of any of SEQ ID NO:3, SEQ ID NO:21, and SEQ ID NO:23. In some embodiments, the CR2-FH is a fusion protein comprising at least about 400, 450, 500, 550, or more contiguous amino acids of any of SEQ ID NO:3, SEQ ID NO:21, and SEQ ID NO:23. In some embodiments, the CR2-FH molecule is a fusion protein encoded by a polynucleotide having nucleic acid sequence of any of SEQ ID NO:4, SEQ ID NO:22, and SEQ ID NO:24. In some embodiments, the CR2-FH molecule is a fusion protein encoded by a polynucleotide having a nucleic acid sequence that is at least about any of 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to that of any of SEQ ID NO:4, SEQ ID NO:22, and SEQ ID NO:24. Also encompassed herein are polynucleotides encoding a CR2-FH fusion protein described herein. For example, in some embodiments, there is provided a polynucleotide encoding a fusion protein comprising a CR2 portion comprising CR2 or a fragment thereof and a FH portion comprising a FH or a fragment thereof. In some embodiments, the polynucleotide also comprises a sequence encoding a signal peptide operably linked at the 5' end of the sequence encoding the CR2-FH fusion protein. In some embodiments, a linker sequence is used for linking the CR2 portion and the FH portion. In some embodiments, the polynucleotide encodes a CR2-FH fusion protein having an amino acid sequence of any of SEQ ID NO:3, SEQ ID NO:21, and SEQ ID NO:23. In some embodiments, the polynucleotide encodes a CR2-FH fusion protein having an amino acid sequence that is at least about any of 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleic acid sequence of any of SEQ ID NO:3, SEQ ID NO:22, and SEQ ID NO:24. Also provided are vectors comprising a polynucleotide encoding a CR2-FH fusion protein, host cells comprising the polynucleotide, and methods of producing a CR2-FH fusion protein comprising culturing the host cells under suitable conditions to express the fusion protein and recovering the fusion protein from the host cell culture.

In another aspect, there is provided a pharmaceutical composition comprising a CR2-FH molecule and a pharmaceutically acceptable carrier. In some embodiments, the pharmaceutical composition comprises a CR2-FH molecule and a pharmaceutically acceptable carrier suitable for administra-

tion to a human. In some embodiments, the pharmaceutical composition comprises a CR2-FH molecule and a pharmaceutically acceptable carrier suitable for intraocular injection. In some embodiments, the pharmaceutical composition comprises a CR2-FH molecule and a pharmaceutically acceptable carrier suitable for topical application to the eye. In some embodiments, the pharmaceutical composition comprises a CR2-FH molecule and a pharmaceutically acceptable carrier suitable for intravenous injection. In some embodiments, the pharmaceutical composition comprises a CR2-FH molecule and a pharmaceutically acceptable carrier suitable for injection into the arteries (such as renal arteries), liver, or kidney.

In some embodiments, the pharmaceutical composition comprises a CR2-FH molecule (such as a CR2 fusion protein) comprising: a) a CR2 portion comprising a CR2 or a fragment thereof, and b) a FH portion comprising a FH or a fragment thereof, and a pharmaceutically acceptable carrier. In some embodiments, the CR2-FH molecule is capable of binding to a CR2 ligand and inhibiting complement activation of the alternative pathway. In some embodiments, the pharmaceutical composition comprises a CR2-FH molecule comprising: a CR2-FH molecule (such as a CR2-FH fusion protein) comprising: a) a CR2 portion comprising at least the first two N-terminal SCR domains of CR2, and b) a FH portion comprising at least the first four N-terminal SCR domains of FH, and a pharmaceutically acceptable carrier. In some embodiments, the pharmaceutical composition comprises a CR2-FH molecule (such as a CR2-FH fusion protein) comprising (and in some embodiments consists of or consists essentially of): a) a CR2 portion comprising the first four N-terminal SCR domains of CR2, and b) a FH portion comprising the first five N-terminal SCR domains of FH, and a pharmaceutically acceptable carrier. In some embodiments, the pharmaceutical composition comprises a CR2-FH molecule (such as a CR2-FH fusion protein) comprising (and in some embodiments consists of or consists essentially of): a) a CR2 portion comprising amino acids 23 to 271 of SEQ ID NO:1, and b) a FH portion comprising amino acids 21 to 320 of SEQ ID NO:2, and a pharmaceutically acceptable carrier. In some embodiments, the pharmaceutical composition comprises a CR2-FH fusion protein having an amino acid sequence that is at least about 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to that of any of SEQ ID NO:3, SEQ ID NO:21, and SEQ ID NO:23, and a pharmaceutically acceptable carrier. In some embodiments, the pharmaceutical composition is suitable for delivery to the eye (for example by intraocular injection or by topical delivery to the eye). In some embodiments, the pharmaceutical composition is suitable for intravenous injection. In some embodiments, the pharmaceutical composition is suitable for injection into arteries (such as renal arteries), liver, or kidney. In some embodiments, the composition is suitable for intraocular, intravenous, intraarterial, sub-cutaneous, intratracheal, or inhalational administration.

In another aspect, the invention provides a method of treating a disease in which the alternative complement pathway is implicated in an individual, comprising administering to the individual an effective amount of a composition (such as a pharmaceutical composition) described herein. In some embodiments, the method comprises administering to the individual an effective amount of a composition comprising a CR2-FH molecule comprising: a) a CR2 portion comprising a CR2 or a fragment thereof, and b) a FH portion comprising a FH or a fragment thereof. In some embodiments, the method comprises administering to the individual an effective amount of a composition comprising a CR2-FH molecule comprising: a) a CR2 portion comprising a CR2 or a fragment

thereof, and b) a FH portion comprising a FH or a fragment thereof, wherein the CR2-FH molecule is capable of binding to a CR2 ligand and wherein the CR2-FH molecule is capable of inhibiting complement activation of the alternative pathway. In some embodiments, the disease to be treated is a disease that involves local inflammation. In some embodiments, the disease to be treated is a disease that is associated with FH deficiencies (including for example decrease in level of FH, decrease in activity of FH, or lacking wildtype or protective FH). In some embodiments, the disease to be treated is not a disease that is associated with FH deficiencies. In some embodiments, the disease to be treated is a drusen-associated disease. In some embodiments, the disease to be treated does not involve the classical complement pathway.

In some embodiments, there is provided a method of treating macular degeneration (such as age-related macular degeneration or AMD) in an individual, comprising administering to the individual an effective amount of a composition comprising a CR2-FH molecule comprising: a) a CR2 portion comprising a CR2 or a fragment thereof, and b) a FH portion comprising a FH or a fragment thereof. In some embodiments, the disease to be treated is a dry form of AMD. In some embodiments, the disease to be treated is a wet form of AMD. In some embodiments, the CR2-FH molecule is administered by intravenous administration. In some embodiments, the CR2-FH molecule is administered by intraocular injection. In some embodiments, the CR2-FH molecule is administered by topical administration to the eye (for example in the form of eye drops).

In some embodiments, one or more aspects of AMD are treated by methods of the present invention. For example, in some embodiments, there is provided a method of treating (such as reducing, delaying, eliminating, or preventing) formation of drusen in the eye of an individual, comprising administering to the individual an effective amount of a composition comprising a CR2-FH molecule comprising: a) a CR2 portion comprising a CR2 or a fragment thereof, and b) a FH portion comprising a FH or a fragment thereof. In some embodiments, there is provided a method of treating (such as reducing, delaying, eliminating, or preventing) inflammation in the eye of an individual, comprising administering to the individual an effective amount of a composition comprising a CR2-FH molecule comprising: a) a CR2 portion comprising a CR2 or a fragment thereof, and b) a FH portion comprising a FH or a fragment thereof. In some embodiments, there is provided a method of treating (such as reducing, delaying, eliminating, or preventing) loss of photoreceptors cells in an individual, comprising administering to the individual an effective amount of a composition comprising a CR2-FH molecule comprising: a) a CR2 portion comprising a CR2 or a fragment thereof, and b) a FH portion comprising a FH or a fragment thereof. In some embodiments, there is provided a method of improving (including for example decreasing, delaying, or blocking loss of) visual acuity or visual field in the eye of an individual, comprising administering to the individual an effective amount of a composition comprising a CR2-FH molecule comprising: a) a CR2 portion comprising a CR2 or a fragment thereof, and b) a FH portion comprising a FH or a fragment thereof. In some embodiments, there is provided a method of treating neovascularization (such as choroidal neovascularization or CNV), comprising administering to the individual an effective amount of a composition comprising a CR2-FH molecule comprising: a) a CR2 portion comprising a CR2 or a fragment thereof, and b) a FH portion comprising a FH or a fragment thereof. Treatments of other aspects of AMD are also contemplated.

The methods described herein are also useful for treatment of certain renal diseases. For example, in some embodiments, there is provided a method of treating membranoproliferative glomerulonephritis type II (MPGN II), comprising administering to the individual an effective amount of a composition comprising a CR2-FH molecule comprising: a) a CR2 portion comprising a CR2 or a fragment thereof, and b) a FH portion comprising a FH or a fragment thereof. In some embodiments, there is provided a method of treating hemolytic-uremic syndrome (HUS), comprising administering to the individual an effective amount of a composition comprising a CR2-FH molecule comprising: a) a CR2 portion comprising a CR2 or a fragment thereof, and b) a FH portion comprising a FH or a fragment thereof. In some embodiments, there is provided a method of treating lupus nephritis, comprising administering to the individual an effective amount of a composition comprising a CR2-FH molecule comprising: a) a CR2 portion comprising a CR2 or a fragment thereof, and b) a FH portion comprising a FH or a fragment thereof.

In some embodiments, there is provided a method of treating ischemia reperfusion (including for example renal ischemia reperfusion and intestinal ischemia reperfusion), comprising administering to the individual an effective amount of a composition comprising a CR2-FH molecule comprising: a) a CR2 portion comprising a CR2 or a fragment thereof, and b) a FH portion comprising a FH or a fragment thereof.

Also provided are methods of treating organ transplant rejections. For example, in some embodiments, there is provided a method of delaying onset of acute vascular rejection (such as antibody-mediated rejection of heart transplant) in an individual, comprising administering to the individual an effective amount of a composition comprising a CR2-FH molecule comprising: a) a CR2 portion comprising a CR2 or a fragment thereof, and b) a FH portion comprising a FH or a fragment thereof.

In some embodiments, there is provided a method of improving organ transplant survival in an individual, comprising administering to the individual an effective amount of a composition comprising a CR2-FH molecule comprising: a) a CR2 portion comprising a CR2 or a fragment thereof, and b) a FH portion comprising a FH or a fragment thereof. In some embodiments, there is provided a method of improving organ transplant survival in an individual, the method comprises perfusing the organ to be transplanted to an individual with a composition comprising a CR2-FH molecule comprising: a) a CR2 portion comprising a CR2 or a fragment thereof, and b) a FH portion comprising a FH or a fragment thereof. In some embodiments, there is provided a method of improving survival of an organ transplant donor, comprising administering to the organ transplant donor an effective amount of a composition comprising a CR2-FH molecule comprising: a) a CR2 portion comprising a CR2 or a fragment thereof, and b) a FH portion comprising a FH or a fragment thereof.

In some embodiments, there is provided a method of treating rheumatoid arthritis, comprising administering to the individual an effective amount of a composition comprising a CR2-FH molecule comprising: a) a CR2 portion comprising a CR2 or a fragment thereof, and b) a FH portion comprising a FH or fragment thereof.

Also provided are unit dosage forms, kits, and articles of manufacture that are useful for methods described herein.

It is to be understood that one, some, or all of the properties of the various embodiments described herein may be combined to form other embodiments of the present invention.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 provides schematic diagrams of an exemplary CR2-FH expression plasmid and CR2-FH proteins. For the CR2-

FH expression plasmid, k refers to Kozak sequence, 5 refers to CD5 signal peptide, 1 refers to an optional linker, s refers to stop codon and polyA signal. For the CR2-FH proteins (with or without signal peptide), 5 refers to the CD5 signal peptide, 1 refers to an optional linker.

FIG. 2 provides the amino acid sequence of human CR2 (SEQ ID NO:1) and the amino acid sequence of human FH (SEQ ID NO:2).

FIG. 3 provides the amino acid sequence of an exemplary human CR2-FH fusion protein (SEQ ID NO: 3) and an exemplary polynucleotide sequence encoding a human CR2-FH fusion protein (SEQ ID NO:4).

FIGS. 4-6 provide exemplary amino acid sequences of CR2-FH molecules described herein (SEQ ID NOs: 5-10). "nnn" represents an optional linker.

FIG. 7 provides exemplary amino acid sequences of signaling peptides described herein (SEQ ID NOs: 11, 13, and 25) and exemplary polynucleotide sequences encoding the signaling peptides (SEQ ID NOs:12, 14, and 26).

FIG. 8 provides amino acid sequence of mouse CR2 (SEQ ID NO:15) and amino acid sequence of mouse FH (SEQ ID NO:16).

FIG. 9 provides amino acid sequence of an exemplary mouse CR2-FH fusion protein (SEQ ID NO:17) and an exemplary polynucleotide sequence that encodes a mouse CR2-FH plus the signal peptide (SEQ ID NO:18).

FIG. 10 provides an exemplary DNA sequence of CR2NLFHFH, a mouse CR2-FH fusion protein containing a CR2 portion and two FH portions without a linker sequence (SEQ ID NO:19).

FIG. 11 provides an exemplary DNA sequence of CR2LFHFH, a mouse CR2-FH fusion protein containing a CR2 portion linked to two FH portions via a linker sequence (SEQ ID NO:20).

FIG. 12A provides a graphic representation of data obtained in an in vitro zymosan complement assay using a mouse CR2-FH fusion protein (CR2-fH) and factor H alone (fH). FIG. 12B provides a graphic representation of data obtained in an in vitro zymosan complement assay using the first five SCR domains of FH (FH 15) and the first four domains of CR2 (CR2).

FIG. 13 provides a graphic representation of data obtained in an in vitro zymosan complement assay using mouse CR2-FH fusion protein with linker (CR2LFH), CR2-FH fusion protein without linker (CR2NLFH), CR2-FH-FH with linker (CR2LFHFH), and CR2-Crry.

FIGS. 14A and 14B provide graphic representations of data obtained in an animal model of intestine ischemia and reperfusion injury using mouse CR2-FH fusion protein having one FH portion (CR2-fH) or two FH portions (CR2-fHH).

FIG. 15A provides a graphic representation of effects of CR2-fH on kidney function as measured by serum urea nitrogen (SUN). FIG. 15B provides a graphic representation of effects of CR2-fH on renal morphology. FIG. 15C and 15D provide immunofluorescence staining results of control mouse (15C) and CR2-fH treated mouse (15D) kidney sections incubated with FTIC-conjugated antibody to mouse C3.

FIG. 16 provides a- and b-wave retinal response results in mice treated with or without CR2-fH.

FIGS. 17A and 17B provides isolectin-b staining of lesions of mouse retina from control mouse (17A) and mouse treated with CR2-fH by intravenous injection (17B). FIG. 17C show quantification of lesion sizes based on the isolectin-b staining of FIGS. 17A and 17B.

FIGS. 18A and 18B provides isolectin-b staining of lesions of mouse retina from control mouse (18A) and mouse treated with CR2-fH by intraoptical injection (18B). FIG. 18C pro-

vides quantification of lesion sizes based on the isolectin-b staining of FIGS. 18A and 18B.

FIG. 19 provides a survival curve of mouse heart transplant recipient treated with single dose of CR2-fH (CR2-fH), multiple doses of CR2-fH (CR2-fH (m)), and control buffer (PBS).

FIG. 20 provides amino acid sequence of an exemplary human CR2-FH fusion protein (designated as human CR2-fH or CR2fH) (SEQ ID NO:21) and an exemplary polynucleotide sequence that encodes a human CR2-fH plus the signal peptide (SEQ ID NO:22). Sequence encoding the signal peptide is underlined.

FIG. 21 provides an exemplary amino acid sequence of a human CR2-FH fusion protein containing two FH portions (designated as human CR2-FH2 or human CR2fH2) (SEQ ID NO:23) and an exemplary polynucleotide sequence that encodes a human CR2-FH2 plus the signal peptide (SEQ ID NO:24). Sequence encoding the signal peptide is underlined.

FIG. 22A shows inhibition of human CR2fH and CR2fH2 on alternative pathway specific C3b deposition onto zymosan particles. FIG. 22B shows inhibition of alternative pathway-mediated erythrocyte lysis by human CR2fH and human CR2fH2.

FIG. 23 shows the effects of mouse CR2-FH on C3 activation induced by immune-complexes of collagen-anti-collagen antibodies. The Y-axis shows mean OD values.

FIG. 24 shows titration of mouse CR2-FH in calcium sufficient buffer using serum from C4-/C4- knockout mouse. The Y-axis shows mean OD values.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a CR2-FH molecule, compositions (such as pharmaceutical compositions) comprising a CR2-FH molecule, and methods of treating a disease in which the alternative complement pathway is implicated by administering the composition. The CR2-FH molecule comprises a CR2 portion and a FH portion. The CR2 portion is responsible for targeted delivery of the molecule to the sites of complement activation, and the FH portion is responsible for specifically inhibiting complement activation of the alternative pathway. Preliminary studies have shown that a CR2-FH molecule, specifically, a CR2-FH fusion protein containing the first four N-terminal SCR domains of the CR2 protein and the first five N-terminal SCR domains the factor H protein, has both targeting activity and complement inhibitory activity in vitro. This molecule is significantly more effective than a factor H molecule lacking the CR2 portion, suggesting that targeting FH to complement activation sites will be an effective therapeutic tool in treating disease in which the alternative complement pathway is implicated, such as macular degeneration (for example age-related macular degeneration). This observation is surprising because of the relatively high concentration of FH in the plasma and the long-held belief that cells which are in direct contact with plasma are already completely covered with FH. Jozsi et al., *Histopathol.* (2004) 19:251-258.

Accordingly, in one aspect, there is provided a CR2-FH molecule comprising: a) a CR2 portion comprising a CR2 or a fragment thereof, and b) a FH portion comprising a FH or a fragment thereof. In some embodiments, there is provided an isolated CR2-FH molecule. In some embodiments, there is provided a composition (such as a pharmaceutical composition) comprising a CR2-FH molecule. For example, in some embodiments, there is provided a pharmaceutical composition comprising a CR2-FH molecule and a pharmaceutically acceptable carrier suitable for administration to an individual

systemically (such as intravenous injection), or locally (such as intraocular injection or injection into arteries including renal arteries).

In another aspect, there is provided a method of treating a disease in which the alternative complement pathway is implicated in an individual, comprising administering to the individual an effective amount of a composition comprising a CR2-FH molecule comprising: a) a CR2 portion comprising a CR2 or a fragment thereof, and b) a FH portion comprising a FH or a fragment thereof. Suitable diseases that can be treated by methods of the present invention include, for example, macular degeneration (such as age-related macular degeneration), rheumatoid arthritis, ischemia reperfusion, organ transplant rejection, and renal diseases such as MPGN II, HUS, and lupus nephritis.

Also provided are unit dosage forms, kits, and articles of manufacture that are useful for methods described herein.

General reference to “the composition” or “compositions” includes and is applicable to compositions of the invention.

As used herein, the singular form “a”, “an”, and “the” includes plural references unless indicated otherwise. For example, “a” FH portion includes one or more FH portions.

Reference to “about” a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se. For example, description referring to “about X” includes description of “X”.

It is understood that aspects and embodiments of the invention described herein include “consisting” and/or “consisting essentially of” aspects and embodiments.

CR2-FH Molecules and Compositions Comprising a CR2-FH Molecule

Provided herein are CR2-FH molecules and compositions (such as pharmaceutical compositions) comprising a CR2-FH molecule.

“CR2-FH molecule” used herein refers to a non-naturally occurring molecule comprising a CR2 or a fragment thereof (the “CR2 portion”) and a FH or a fragment thereof (the “FH portion”). The CR2 portion is capable of binding to one or more natural ligands of CR2 and is thus responsible for targeted delivery of the molecule to the sites of complement activation. The FH portion is responsible for specifically inhibiting complement activation of the alternative complement pathway. The CR2 portion and the FH portion of the CR2-FH molecule can be linked together by any methods known in the art, as long as the desired functionalities of the two portions are maintained.

The CR2-FH molecule described herein thus generally has the dual functions of binding to a CR2 ligand and inhibiting complement activation of the alternative pathway. “CR2 ligand” refers to any molecule that binds to a naturally occurring CR2 protein, which include, but are not limited to, C3d, iC3b, C3dg, C3d, and cell-bound fragments of C3b that bind to the two N-terminal SCR domains of CR2. The CR2-FH molecule may, for example, bind to a CR2 ligand with a binding affinity that is about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of the CR2 protein. Binding affinity can be determined by any method known in the art, including for example, surface plasmon resonance, calorimetry titration, ELISA, and flow cytometry. In some embodiments, the CR2-FH molecule has one or more of the following properties of CR2: (1) binding to C3d, (2) binding to iC3b, (3) binding to C3dg, (4) binding to cell-bound fragment(s) of C3b that bind to the two N-terminal SCR domains of CR2.

The CR2-FH molecule described herein is generally capable of inhibiting complement activation of the alternative pathway. The CR2-FH molecule may be a more potent

complement inhibitor than the naturally occurring FH protein. For example, in some embodiments, the CR2-FH molecule has a complement inhibitory activity that is about any of 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 25, 30, 40, or more fold of that of the FH protein. In some embodiments, the CR2-FH molecule has an EC50 of less than about any of 100 nM, 90 nM, 80 nM, 70 nM, 60 nM, 50 nM, 40 nM, 30 nM, 20 nM, or 10 nM. In some embodiments, the CR2-FH molecule has an EC50 of about 5-60 nM, including for example any of 8-50 nM, 8-20 nM, 10-40 nM, and 20-30 nM. In some embodiments, the CR2-FH molecule has complement inhibitory activity that is about any of 50%, 60%, 70%, 80%, 90%, or 100% of that of the FH protein.

Complement inhibition can be evaluated based on any methods known in the art, including for example, in vitro zymosan assays, assays for lysis of erythrocytes, immune complex activation assays, and mannan activation assays. In some embodiments, the CR2-FH has one or more of the following properties of FH: (1) binding to C-reactive protein (CRP), (2) binding to C3b, (3) binding to heparin, (4) binding to sialic acid, (5) binding to endothelial cell surfaces, (6) binding to cellular integrin receptor, (7) binding to pathogens, (8) C3b co-factor activity, (9) C3b decay-acceleration activity, and (10) inhibiting the alternative complement pathway.

In some embodiments, the CR2-FH molecule is a fusion protein. “Fusion protein” used herein refers to two or more peptides, polypeptides, or proteins operably linked to each other. In some embodiments, the CR2 portion and the FH portion are directly fused to each other. In some embodiments, the CR2 portion and the FH portion are linked by an amino acid linker sequence. Examples of linker sequences are known in the art, and include, for example, (Gly₄Ser), (Gly₄Ser)₂, (Gly₄Ser)₃, (Gly₃Ser)₄, (SerGly₄), (SerGly₄)₂, (SerGly₄)₃, and (SerGly₄)₄. Linking sequences can also comprise “natural” linking sequences found between different domains of complement factors. For example, VSVFPLE, the linking sequence between the first two N-terminal short consensus repeat domains of human CR2, can be used. In some embodiments, the linking sequence between the fourth and the fifth N-terminal short consensus repeat domains of human CR2 (EEIF) is used. The order of CR2 portion and FH portion in the fusion protein can vary. For example, in some embodiments, the C-terminus of the CR2 portion is fused (directly or indirectly) to the N-terminus of the FH portion of the molecule. In some embodiments, the N-terminus of the CR2 portion is fused (directly or indirectly) to the C-terminus of the FH portion of the molecule.

In some embodiments, the CR2-FH molecule is a CR2-FH fusion protein having an amino acid sequence of any of SEQ ID NO:3, SEQ ID NO:21, and SEQ ID NO:23. In some embodiments, the CR2-FH molecule is a fusion protein having an amino acid sequence that is at least about 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to that of any of SEQ ID NO:3, SEQ ID NO:21, or SEQ ID NO:23. In some embodiments, the CR2-FH molecule comprises at least about 400, 450, 500, 550, or more contiguous amino acids of any of SEQ ID NO:3, SEQ ID NO:21, and SEQ ID NO:23.

In some embodiments, the CR2-FH molecule is a CR2-FH fusion protein having an amino acid sequence of any of SEQ ID NOs:5-10. In some embodiments, the CR2-FH molecule is a fusion protein having an amino acid sequence that is at least about 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to that of any of SEQ ID NOs:5-10. In some embodiments, the CR2-FH molecule comprises at least about 400, 450, 500, 550, or more contiguous amino acids any of SEQ ID NOs:5-10.

In some embodiments, the CR2-FH molecule is encoded by a polynucleotide having nucleic acid sequence of any of SEQ ID NO:4, SEQ ID NO:22, and SEQ ID NO:24. In some embodiments, the CR2-FH molecule is encoded by a polynucleotide having a nucleic acid sequence that is at least about 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to that of any of SEQ ID NO:4, SEQ ID NO:22, and SEQ ID NO:24.

In some embodiments, the CR2-FH molecule comprises a CR2 portion and a FH portion linked via a chemical cross-linker. Linking of the two portions can occur on reactive groups located on the two portions. Reactive groups that can be targeted using a crosslinker include primary amines, sulfhydryls, carbonyls, carbohydrates, and carboxylic acids, or active groups that can be added to proteins. Examples of chemical linkers are well known in the art and include, but are not limited to, bismaleimido-hexane, maleimidobenzoyl-N-hydroxysuccinimide ester, NHS-Esters-Maleimide Crosslinkers such as SPDP, carbodiimide, glutaraldehyde, MBS, Sulfo-MBS, SMPB, sulfo-SMPB, GMBS, Sulfo-GMBS, EMCS, Sulfo-EMCS, imidoester crosslinkers such as DMA, DMP, DMS, DTBP, EDC and DTME.

In some embodiments, the CR2 portion and the FH portion are non-covalently linked. For example, the two portions may be brought together by two interacting bridging proteins (such as biotin and streptavidin), each linked to a CR2 portion or a FH portion.

In some embodiments, the CR2-FH molecule comprises two or more (same or different) CR2 portions described herein. In some embodiments, the CR2-FH molecule comprises two or more (same or different) FH portions described herein. These two or more CR2 (or FH) portions may be tandemly linked (such as fused) to each other. In some embodiments, the CR2-FH molecule (such a CR2-FH fusion protein) comprises a CR2 portion and two or more (such as three, four, five, or more) FH portions. In some embodiments, the CR2-FH molecule (such a CR2-FH fusion protein) comprises a FH portion and two or more (such as three, four, five, or more) CR2 portions. In some embodiments, the CR2-FH molecule (such a CR2-FH fusion protein) comprises two or more CR2 portions and two or more FH portions.

In some embodiments, there is provided an isolated CR2-FH molecule. In some embodiments, the CR2-FH molecules form dimers or multimers.

The CR2 portion and the FH portion in the molecule can be from the same species (such as human or mouse), or from different species.

CR2 Portion

The CR2 portion described herein comprises a CR2 or a fragment thereof. CR2 is a transmembrane protein expressed predominantly on mature B cells and follicular dendritic cells. CR2 is a member of the C3 binding protein family. Natural ligands for CR2 include, for example, iC3b, C3dg, and C3d, and cell-bound breakdown fragments of C3b that bind to the two N-terminal SCR domains of CR2. Cleavage of C3 results initially in the generation of C3b and the covalent attachment of this C3b to the activating cell surface. The C3b fragment is involved in the generation of enzymatic complexes that amplify the complement cascade. On a cell surface, C3b is rapidly converted to inactive iC3b, particularly when deposited on a host surface containing regulators of complement activation (i.e., most host tissue). Even in absence of membrane bound complement regulators, substantial levels of iC3b are formed. iC3b is subsequently digested to the membrane bound fragments C3dg and then C3d by serum proteases, but this process is relatively slow. Thus, the C3 ligands for CR2 are relatively long lived once

they are generated and will be present in high concentrations at sites of complement activation. CR2 therefore can serve as a potent targeting vehicle for bringing molecules to the site of complement activation.

CR2 contains an extracellular portion having 15 or 16 repeating units known as short consensus repeats (SCR domains). The SCR domains have a typical framework of highly conserved residues including four cysteines, two prolines, one tryptophane and several other partially conserved glycines and hydrophobic residues. SEQ ID NO:1 represents the full-length human CR2 protein sequence. Amino acids 1-20 comprise the leader peptide, amino acids 23-82 comprise SCR1, amino acids 91-146 comprise SCR2, amino acids 154-210 comprise SCR3, amino acids 215-271 comprise SCR4. The active site (C3d binding site) is located in SCR1-2 (the first two N-terminal SCR domains). These SCR domains are separated by short sequences of variable length that serve as spacers. The full-length mouse CR2 protein sequence is represented herein by SEQ ID NO:15. The SCR1 and SCR2 domains of the mouse CR2 protein are located with the mouse CR2 amino sequence at positions 14-73 of SEQ ID NO:15 (SCR1) and positions 82-138 of SEQ ID NO:15 (SCR2). Human and mouse CR2 are approximately 66% identical over the full length amino acid sequences represented by SEQ ID NO:1 and SEQ ID NO:15, and approximately 61% identical over the SCR1-SCR2 regions of SEQ ID NO:1 and SEQ ID NO:15. Both mouse and human CR2 bind to C3 (in the C3d region). It is understood that species and strain variations exist for the disclosed peptides, polypeptides, and proteins, and that the CR2 or a fragment thereof described herein encompasses all species and strain variations.

The CR2 portion disclosed herein refers to a polypeptide that contains some or all of the ligand binding sites of the CR2 protein, and includes, but is not limited to, full-length CR2 proteins (such as human CR2 as shown in SEQ ID NO:1 or mouse CR2 as shown in SEQ ID NO:15), soluble CR2 proteins (such as a CR2 fragment comprising the extracellular domain of CR2), other biologically active fragments of CR2, a CR2 fragment comprising SCR1 and SCR2, or any homologue of a naturally occurring CR2 or fragment thereof, as described in detail below. In some embodiments, the CR2 portion has one of the following properties or CR2: (1) binding to C3d, (2) binding to iC3b, (3) binding to C3dg, (4) binding to cell-bound fragment(s) of C3b that bind to the two N-terminal SCR domains of CR2.

In some embodiments, the CR2 portion comprises the first two N-terminal SCR domains of CR2. In some embodiments, the CR2 portion comprises the first three N-terminal SCR domains of CR2. In some embodiments, the CR2 portion comprises the first four N-terminal SCR domains of CR2. In some embodiments, the CR2 portion comprises (and in some embodiments consists of or consists essentially of) at least the first two N-terminal SCR domains of CR2, including for example at least any of the first 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 SCR domains of CR2.

A homologue of a CR2 protein or a fragment thereof includes proteins which differ from a naturally occurring CR2 (or CR2 fragment) in that at least one or a few amino acids have been deleted (e.g., a truncated version of the protein, such as a peptide or fragment), inserted, inverted, substituted and/or derivatized (e.g., by glycosylation, phosphorylation, acetylation, myristoylation, prenylation, palmitation, amidation and/or addition of glycosylphosphatidylinositol). In some embodiments, a CR2 homologue has an amino acid sequence that is at least about 70% identical to the amino acid sequence of a naturally occurring CR2 (e.g., SEQ

ID NO:1, or SEQ ID NO:15), for example at least about any of 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of a naturally occurring CR2 (e.g., SEQ ID NO:1, or SEQ ID NO:15). A CR2 homologue or a fragment thereof preferably retains the ability to bind to a naturally occurring ligand of CR2 (e.g., C3d or other C3 fragments with CR2-binding ability). For example, the CR2 homologue (or fragment thereof) may have a binding affinity for C3d that is at least about 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of that of CR2 (or a fragment thereof).

In some embodiments, the CR2 portion comprises at least the first two N-terminal SCR domains of a human CR2, such as a CR2 portion having an amino acid sequence containing at least amino acids 23 through 146 of the human CR2 (SEQ ID NO:1). In some embodiments, the CR2 portion comprises at least the first two SCR domains of human CR2 having an amino acid sequence that is at least about any of 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to amino acids 23 through 146 of the human CR2 (SEQ ID NO:1).

In some embodiments, the CR2 portion comprises at least the first four N-terminal SCR domains of a human CR2, such as a CR2 portion having an amino acid sequence containing at least amino acids 23 through 271 of the human CR2 (SEQ ID NO:1). In some embodiments, the CR2 portion comprises at least the first four SCR domains of human CR2 having an amino acid sequence that is at least about any of 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to amino acids 23 through 271 of the human CR2 (SEQ ID NO:1).

An amino acid sequence that is at least about, for example, 95% identical to a reference sequence (such as SEQ ID NO:1) is intended that the amino acid sequence is identical to the reference sequence except that the amino acid sequence may include up to five point alterations per each 100 amino acids of the reference sequence. These up to five point alterations may be deletions, substitutions, additions, and may occur anywhere in the sequence, interspersed either individually among amino acids in the reference sequence or in one or more continuous groups within the reference sequence.

In some embodiments, the CR2 portion comprises part or all of the ligand binding sites of the CR2 protein. In some embodiments, the CR2 portion further comprises sequences required to maintain the three dimensional structure of the binding site. Ligand binding sites of CR2 can be readily determined based on the crystal structures of CR2, such as the human and mouse CR2 crystal structures disclosed in U.S. Patent Application Publication No. 2004/0005538. For example, in some embodiments, the CR2 portion comprises the B strand and B-C loop of SCR2 of CR2. In some embodiments, the CR2 portion comprises a site on strand B and the B-C loop of CR2 SCR comprising the segment G98-G99-Y100-K101-I102-R103-G104-S105-T106-P107-Y108 with respect to SEQ ID NO: 1. In some embodiments, the CR2 portion comprises a site on the B strand of CR2 SCR2 comprising position K119 with respect to SEQ ID NO:1. In some embodiments, the CR2 portion comprises a segment comprising V149-F150-P151-L152, with respect to SEQ ID NO:1. In some embodiments, the CR2 portion comprises a segment of CR2 SCR2 comprising T120-N121-F122. In some embodiments, the CR2-FH molecule has two or more of these sites.

For example, in some embodiments, the CR2 portion comprises a portion comprising G98-G99-Y100-K101-I102-R103-G104-5105-T106-P107-Y108 and K119 with respect to SEQ ID NO:1. Other combinations of these sites are also contemplated.

Factor H Portion

The FH portion of the CR2-FH molecule described herein comprises a FH or a fragment thereof.

Complement factor H (FH) is a single polypeptide chain plasma glycoprotein. The protein is composed of 20 repetitive SCR domains of approximately 60 amino acids, arranged in a continuous fashion like a string of 20 beads. Factor H binds to C3b, accelerates the decay of the alternative pathway C3-converterase (C3Bb), and acts as a cofactor for the proteolytic inactivation of C3b. In the presence of factor H, C3b proteolysis results in the cleavage of C3b. Factor H has at least three distinct binding domains for C3b, which are located within SCR 1-4, SCR 5-8, and SCR 19-20. Each site of factor H binds to a distinct region within the C3b protein: the N-terminal sites bind to native C3b; the second site, located in the middle region of factor H, binds to the C3c fragment and the site located within SCR19 and 20 binds to the C3d region. In addition, factor H also contains binding sites for heparin, which are located within SCR 7, SCR 5-12, and SCR20 of factor H and overlap with that of the C3b binding site. Structural and functional analyses have shown that the domains for the complement inhibitory activity of FH are located within the first four N-terminal SCR domains.

SEQ ID NO:2 represents the full-length human FH protein sequence. Amino acids 1-18 correspond to the leader peptide, amino acids 21-80 correspond to SCR1, amino acids 85-141 correspond to SCR2, amino acids 146-205 correspond to SCR3, amino acids 210-262 correspond to SCR4, amino acids 267-320 correspond to SCR5. The full-length mouse FH protein sequence is represented herein by SEQ ID NO:16. The SCR1 and SCR2 domains of the mouse FH protein are located with the mouse FH amino sequence at positions 21-27 of SEQ ID NO:16 (SCR1) and positions 82-138 of SEQ ID NO:16 (SCR2). Human and mouse FH are approximately 61% identical over the full length amino acid sequences represented by SEQ ID NO:2 and SEQ ID NO:16. It is understood that species and strain variations exist for the disclosed peptides, polypeptides, and proteins, and that the FH or a fragment thereof encompasses all species and strain variations.

The FH portion described herein refers to any portion of a FH protein having some or all the complement inhibitory activity of the FH protein, and includes, but is not limited to, full-length FH proteins, biologically active fragments of FH proteins, a FH fragment comprising SCR1-4, or any homologue of a naturally occurring FH or fragment thereof, as described in detail below. In some embodiments, the FH portion has one or more of the following properties: (1) binding to C-reactive protein (CRP), (2) binding to C3b, (3) binding to heparin, (4) binding to sialic acid, (5) binding to endothelial cell surfaces, (6) binding to cellular integrin receptor, (7) binding to pathogens, (8) C3b co-factor activity, (9) C3b decay-acceleration activity, and (10) inhibiting the alternative complement pathway.

In some embodiments, the FH portion comprises the first four N-terminal SCR domains of FH. In some embodiments, the construct comprises the first five N-terminal SCR domains of FH. In some embodiments, the construct comprises the first six N-terminal SCR domains of FH. In some embodiments, the FH portion comprises (and in some embodiments consists of or consisting essentially of) at least the first four N-terminal SCR domains of FH, including for

example, at least any of the first 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or more N-terminal SCR domains of FH.

In some embodiments, the FH is a wildtype FH. In some embodiments, the FH is a protective variant of FH.

In some embodiments, the FH portion lacks a heparin binding site. This can be achieved, for example, by mutation of the heparin binding site on FH, or by selecting FH fragments that do not contain a heparin binding site. In some embodiments, the FH portion comprises a FH or a fragment thereof having a polymorphism that is protective to age-related macular degeneration. Hageman et al., *Proc. Natl. Acad. Sci. USA* 102(20):7227. One example of a CR2-FH molecule comprising such a sequence is provided in FIG. 4 (SEQ ID NO:6).

A homologue of a FH protein or a fragment thereof includes proteins which differ from a naturally occurring FH (or FH fragment) in that at least one or a few, but not limited to one or a few, amino acids have been deleted (e.g., a truncated version of the protein, such as a peptide or fragment), inserted, inverted, substituted and/or derivatized (e.g., by glycosylation, phosphorylation, acetylation, myristoylation, prenylation, palmitation, amidation and/or addition of glycosylphosphatidyl inositol). For example, a FH homologue may have an amino acid sequence that is at least about 70% identical to the amino acid sequence of a naturally occurring FH (e.g., SEQ ID NO:2, or SEQ ID NO:16), for example at least about any of 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of a naturally occurring FH (e.g., SEQ ID NO:2, or SEQ ID NO:16). In some embodiment, a homologue of FH (or a fragment thereof) retains all the complement inhibition activity of FH (or a fragment thereof). In some embodiments, the homologue of FH (or a fragment thereof) retains at least about 50%, for example, at least about any of 60%, 70%, 80%, 90%, or 95% of the complement inhibition activity of FH (or a fragment thereof).

In some embodiments, the FH portion comprises at least the first four N-terminal SCR domains of a human FH, such as a FH portion having an amino acid sequence containing at least amino acids 21 through 262 of the human FH (SEQ ID NO:2). In some embodiments, the FH portion comprises at least the first four N-terminal SCR domains of human FH having an amino acid sequence that is at least about any of 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to amino acids 21 through 262 of the human FH (SEQ ID NO:2).

In some embodiments, the FH portion comprises at least the first five N-terminal SCR domains of a human FH, such as a FH portion having an amino acid sequence containing at least amino acids 21 through 320 of the human FH (SEQ ID NO:2). In some embodiments, the FH portion comprises at least the first five N-terminal SCR domains of human FH having an amino acid sequence that is at least about any of 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to amino acids 21 through 320 of the human FH (SEQ ID NO:2).

In some embodiments, the FH portion comprises a full length or a fragment of factor-H like 1 molecule (FHL-1), a protein encoded by an alternatively spliced transcript of the factor H gene. The mature FHL-1 contains 431 amino acids. The first 427 amino acids organize seven SCR domains and are identical to the N-terminal SCR domains of FH. The remaining four amino acid residues Ser-Phe-Thr-Leu (SFTL) at the C-terminus are specific to FHL-1. FHL-1 has been

characterized functionally and shown to have factor H complement regulatory activity. The term "FH portion" also encompasses full length or fragments of factor H related molecules, including, but are not limited to, proteins encoded by the FHR1, FHR2, FHR3, FHR4, FHR5 genes. These factor H related proteins are disclosed, for example, in de Cordoba et al., *Molecular Immunology* 2004, 41:355-367.

Variants of CR2-FH Molecules

Also encompassed are variants of the CR2-FH molecules (such as the CR2-FH fusion proteins). A variant of the CR2-FH molecule described herein may be: (i) one in which one or more of the amino acid residues of the CR2 portion and/or the FH portion are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code; or (ii) one in which one or more of the amino acid residues in the CR2 portion and/or FH portion includes a substituent group, or (iii) one in which the CR2-FH molecule (such as the CR2-FH fusion protein) is fused with another compound, such as a compound to increase the half-life of the CR2-FH molecule (for example, polyethylene glycol), or (iv) one in which additional amino acids are fused to the CR2-FH molecule (such as the CR2-FH fusion protein), such as a leader or secretory sequence or a sequence which is employed for purification of the CR2-FH molecule (such as the CR2-FH fusion protein), or (v) one in which the CR2-FH molecule (such as the CR2-FH fusion protein) is fused with a larger polypeptide, i.e., human albumin, an antibody or Fc, for increased duration of effect. Such variants are deemed to be within the scope of those skilled in the art from the teachings herein.

In some embodiments, the variant of the CR2-FH molecule contains conservative amino acid substitutions (defined further below) made at one or more predicted, preferably non-essential amino acid residues. A "nonessential" amino acid residue is a residue that can be altered from the wild-type sequence of a protein without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine).

Amino acid substitutions in the CR2 or FH portions of the CR2-FH molecule can be introduced to improve the functionality of the molecule. For example, amino acid substitutions can be introduced into the CR2 portion of the molecule to increase binding affinity of the CR2 portion to its ligand(s), increase binding specificity of the CR2 portion to its ligand(s), improve targeting of the CR2-FH molecule to desired sites, increase dimerization or multimerization of CR2-FH molecules, and improve pharmacokinetics of the CR2-FH molecule. Similarly, amino acid substitutions can be introduced into the FH portion of the molecule to increase the functionality of the CR2-FH molecule and improve pharmacokinetics of the CR2-FH molecule.

In some embodiments, the CR2-FH molecule (such as the CR2-FH fusion protein) is fused with another compound, such as a compound to increase the half-life of the polypep-

tide and/or to reduce potential immunogenicity of the polypeptide (for example, polyethylene glycol, "PEG"). The PEG can be used to impart water solubility, size, slow rate of kidney clearance, and reduced immunogenicity to the fusion protein. See e.g., U.S. Pat. No. 6,214,966. In the case of PEGylations, the fusion of the CR2-FH molecule (such as the CR2-FH fusion protein) to PEG can be accomplished by any means known to one skilled in the art. For example, PEGylation can be accomplished by first introducing a cysteine mutation into the CR2-FH fusion protein, followed by site-specific derivatization with PEG-maleimide. The cysteine can be added to the C-terminus of the CR2-FH fusion protein. See, e.g., Tsutsumi et al. (2000) *Proc. Natl. Acad. Sci. USA* 97(15):8548-8553. Another modification which can be made to the CR2-FH molecule (such as the CR2-FH fusion protein) involves biotinylation. In certain instances, it may be useful to have the CR2-FH molecule (such as the CR2-FH fusion protein) biotinylated so that it can readily react with streptavidin. Methods for biotinylation of proteins are well known in the art. Additionally, chondroitin sulfate can be linked with the CR2-FH molecule (such as the CR2-FH fusion protein).

In some embodiments, the CR2-FH molecule is fused to another targeting molecule or targeting moiety which further increases the targeting efficiency of the CR2-FH molecule. For example, the CR2-FH molecule can be fused to a ligand (such as an amino acid sequence) that has the capability to bind or otherwise attach to an endothelial cell of a blood vessel (referred to as "vascular endothelial targeting amino acid ligand"). Exemplary vascular endothelial targeting ligands include, but are not limited to, VEGF, FGF, integrin, fibronectin, I-CAM, PDGF, or an antibody to a molecule expressed on the surface of a vascular endothelial cell.

In some embodiments, the CR2-FH molecule is conjugated (such as fused) to a ligand for intercellular adhesion molecules. For example, the CR2-FH molecule can be conjugated to one or more carbohydrate moieties that bind to an intercellular adhesion molecule. The carbohydrate moiety facilitates localization of the CR2-FH molecule to the site of injury. The carbohydrate moiety can be attached to the CR2-FH molecule by means of an extracellular event such as a chemical or enzymatic attachment, or can be the result of an intracellular processing event achieved by the expression of appropriate enzymes. In some embodiments, the carbohydrate moiety binds to a particular class of adhesion molecules such as integrins or selectins, including E-selectin, L-selectin or P-selectin. In some embodiments, the carbohydrate moiety comprises an N-linked carbohydrate, for example the complex type, including fucosylated and sialylated carbohydrates. In some embodiments, the carbohydrate moiety is related to the Lewis X antigen, for example the sialylated Lewis X antigen.

For treatment of eye diseases such as AMD, the CR2-FH can be conjugated (such as fused) to an antibody that recognizes a neoepitope of the drusen. Other targeting molecules such as small targeting peptide can also be used. Other modifications of the CR2-FH molecule include, for example, glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, and the like.

The CR2-FH molecule may include the addition of an immunologically active domain, such as an antibody epitope or other tag, to facilitate targeting or purification of the polypeptide. The use of 6×His and GST (glutathione S transferase) as tags is well known. Inclusion of a cleavage site at or near the fusion junction will facilitate removal of the extraneous polypeptide after purification. Other amino acid sequences that may be included in the CR2-FH molecule

include functional domains, such as active sites from enzymes such as a hydrolase, glycosylation domains, and cellular targeting signals.

Variants of the CR2-FH molecule (such as the CR2-FH fusion protein) include polypeptides having an amino acid sequence sufficiently similar to the amino acid sequence of the CR2-FH molecule. The term "sufficiently similar" means a first amino acid sequence that contains a sufficient or minimum number of identical or equivalent amino acid residues relative to a second amino acid sequence such that the first and second amino acid sequences have a common structural domain and/or common functional activity. For example, amino acid sequences that contain a common structural domain that is at least about 45%, preferably about 75% through 98%, identical are defined herein as sufficiently similar. Variants include variants of fusion proteins encoded by a polynucleotide that hybridizes to a polynucleotide of this invention or a complement thereof under stringent conditions. Such variants generally retain the functional activity of the fusion proteins of this invention. Libraries of fragments of the polynucleotides can be used to generate a variegated population of fragments for screening and subsequent selection. For example, a library of fragments can be generated by treating a double-stranded PCR fragment of a polynucleotide with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double-stranded DNA, renaturing the DNA to form double-stranded DNA which can include sense/antisense pairs from different nicked products, removing single-stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, one can derive an expression library that encodes N-terminal and internal fragments of various sizes of the fusion proteins of this invention.

Variants include fusion proteins that differ in amino acid sequence due to mutagenesis. In addition, bioequivalent analogs of the CR2-FH molecule (such as fusion protein) may also be constructed by making various substitutions on residues or sequences in the CR2 portion and/or the FH portion.

In some embodiments, the CR2-FH molecule, particularly the CR2-FH fusion protein, is fused at its N-terminus a signal peptide. Such signal peptides are useful for the secretion of the CR2-FH molecule. Suitable signal peptides include, for example, the signal peptide of the CD5 protein (such as signal peptide of the human CD5 protein MPMGSLQPLATLYLLGMLVAS, SEQ ID NO:11). In some embodiments, the signal peptide of the CR2 protein is used. For example, in some embodiments, the signal peptide of the human CR2 protein (MGAAGLLGVFLALVAPG, SEQ ID NO:13 or MGAAGLLGVFLALVAPGVLG, SEQ ID NO:25) is used.

Preparation of CR2-FH Molecules

The CR2-FH molecules (or the two portions of the CR2-FH molecules) described herein may be made by chemical synthesis methods, or by linkage of a polynucleotide encoding the CR2 portion and a polynucleotide encoding the FH portion (with or without a linker sequence), and introducing the resulting polynucleotide molecule in a vector for transfecting host cells that are capable of expressing the molecule. Chemical synthesis, especially solid phase synthesis, is preferred for short peptides or those containing unnatural or unusual amino acids such as D-Tyr, Ornithine, and the like. Recombinant procedures are preferred for longer polypeptides. The CR2-FH molecule can be isolated in vitro by protein purification methods. The CR2-FH molecule can also be provided "in situ" by introduction of a gene therapy system to the tissue of interest which then expresses the CR2-FH fusion.

Recombinant DNA techniques for making a CR2-FH fusion protein involves, in simplified form, taking the a CR2-FH encoding polynucleotide, inserting it into an appropriate vector, inserting the vector into an appropriate host cell, and recovering or isolating the fusion protein produced thereby.

Provided herein are polynucleotides that encode a CR2-FH molecule (i.e., a CR2-FH fusion protein). Such polynucleotide may also be used for delivery and expression of CR2-FH. For example, in some embodiments, there is provided a polynucleotide encoding a fusion protein comprising a CR2 portion comprising a CR2 or a fragment thereof and a FH portion comprising a FH or a fragment thereof. In some embodiments, the polynucleotide also comprises a sequence encoding a signal peptide operably linked at the 5' end of the sequence encoding the CR2-FH fusion protein. Exemplary nucleotide sequences of signal peptides are provided in FIG. 7 (SEQ ID NO:12, 14, and 25). In some embodiments, a linker sequence is used for linking the CR2 portion and the FH portion. In some embodiments, the polynucleotide encodes a CR2-FH fusion protein having an amino acid sequence of SEQ ID NO:3. In some embodiments, the polynucleotide encodes a CR2-FH fusion protein having an amino acid sequence that is at least about any of 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of any of SEQ ID NO:3, SEQ ID NO:21, and SEQ ID NO:23. In some embodiments, the polynucleotide encodes a CR2-FH molecule comprising at least about any of 400, 450, 500, 550, or more contiguous nucleotides of any of SEQ ID NO:4, SEQ ID NO:22, and SEQ ID NO:24. In some embodiments, the polynucleotide comprises a sequence any of SEQ ID NO:4, SEQ ID NO:22, and SEQ ID NO:24. In some embodiments, the polynucleotide comprises a sequence that is at least about any of 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleic acid sequence any of SEQ ID NO:4, SEQ ID NO:22, and SEQ ID NO:24. In some embodiments, the polynucleotide comprises least about any of 1200, 1300, 1400, 1500, 1600, or more contiguous nucleotides any of SEQ ID NO:4, SEQ ID NO:22, and SEQ ID NO:24. The polynucleotide may further include a sequence encoding a secretory signal sequence to secrete the fusion protein into a medium. The polynucleotide encoding a secretory signal sequence include, for example, a polynucleotide encoding the signal sequence of CD5 or a polynucleotide sequence encoding the signal sequence of CR2.

Also provided are expression vectors comprising a polynucleotide described herein for expression of the CR2-FH fusion protein. The expression vector can be used to direct expression of a CR2-FH fusion protein in vitro or in vivo. The vector may include any element to establish a conventional function of a vector, for example, promoter, terminator, selection marker, and origin of replication. The promoter can be constitutive or regulative, and is selected from, for example, promoters of genes for galactokinase (GAL1), uridylyltransferase (GALT), epimerase (GAL10), phosphoglycerate kinase (PGK), glyceraldehydes-3-phosphate dehydrogenase (GPD), alcohol dehydrogenase (ADH), and the like.

Many expression vectors are known to those of skill in the art. For example, *E. coli* may be transformed using pBR322, a plasmid derived from an *E. coli* species (Mandel et al., J. Mol. Biol., 53:154 (1970)). Plasmid pBR322 contains genes for ampicillin and tetracycline resistance, and thus provides easy means for selection. Other vectors include different features such as different promoters, which are often important in expression. For example, plamids pKK223-3 (Pharmacia

Fine Chemicals, Uppsala, Sweden), pKK233-2 (Clontech, Palo Alto, Calif., USA), and pGEM1 (Promega Biotech, Madison, Wis., USA), are all commercially available. Other vectors that can be used in the present invention include, but are not limited to, pET21a (Studier et al., *Methods Enzymol.*, 185: 60-89 (1990)), pR1T5, and pR1T2T (Pharmacia Biotechnology), and pB0475 (Cunningham et al., *Science*, 243: 1330-1336 (1989); U.S. Pat. No. 5,580,723). Mammalian expression vectors may contain non-transcribed elements such as an origin of replication, promoter and enhancer, and 5' or 3' nontranslated sequences such as ribosome binding sites, a polyadenylation site, acceptor site and splice donor, and transcriptional termination sequences. Promoters for use in mammalian expression vectors usually are for example viral promoters such as Polyoma, Adenovirus, HTLV, Simian Virus 40 (SV 40), and human cytomegalovirus (CMV). Vectors can also be constructed using standard techniques by combining the relevant traits of the vectors described above.

Also provided are host cells (such as isolated cells, transient cell lines, and stable cell lines) for expressing a CR2-FH fusion protein. The host cell may be prokaryotic or eukaryotes. Exemplary prokaryote host cells include *E. coli* K12 strain 294 (ATCC No. 31446), *E. coli* B, *E. coli* X1776 (ATCC No. 31537), *E. coli* W3110 (F-, gamma-, prototrophic/ATCC No. 27325), bacilli such as *Bacillus subtilis*, and other enterobacteriaceae such as *Salmonella typhimurium* or *Serratia marcesans*, and various *Pseudomonas* species. One suitable prokaryotic host cell is *E. coli* BL21 (Stratagene), which is deficient in the OmpT and Lon proteases, which may interfere with isolation of intact recombinant proteins, and useful with T7 promoter-driven vectors, such as the pET vectors. Another suitable prokaryote is *E. coli* W3110 (ATCC No. 27325). When expressed by prokaryotes the peptides typically contain an N-terminal methionine or a formyl methionine and are not glycosylated. In the case of fusion proteins, the N-terminal methionine or formyl methionine resides on the amino terminus of the fusion protein or the signal sequence of the fusion protein. These examples are, of course, intended to be illustrative rather than limiting.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for fusion-protein-encoding vectors. *Saccharomyces cerevisiae* is a commonly used lower eukaryotic host microorganism. Others include *Schizosaccharomyces pombe* (Beach and Nurse, *Nature*, 290: 140 (1981); EP 139,383 published 2 May 1985); *Kluyveromyces* hosts (U.S. Pat. No. 4,943,529; Fleer et al., *Bio/Technology*, 9:968-975 (1991)) such as, e.g., *K. lactis* (MW98-8C, CBS683, CBS4574; Louvencourt et al., *J. Bacteriol.*, 154(2):737-742 (1983)), *K. fragilis* (ATCC 12,424), *K. bulgaricus* (ATCC No. 16,045), *K. wickerhamii* (ATCC No. 24,178), *K. waltii* (ATCC No. 56,500), *K. drosophilorum* (ATCC No. 36,906; Van den Berg et al., *Bio/Technology*, 8:135 (1990)), *K. thermotolerans*, and *K. marxianus*; yarrowia (EP 402,226); *Pichia pastoris* (EP 183,070; Sreekrishna et al., *J. Basic Microbiol.*, 28:265-278 (1988)); *Candida*; *Trichoderma reesia* (EP 244,234); *Neurospora crassa* (Case et al., *Proc. Natl. Acad. Sci. USA*, 76:5259-5263 (1979)); *Schwanniomyces* such as *Schwanniomyces occidentalis* (EP 394,538 published 31 Oct. 1990); and filamentous fungi such as, e.g., *Neurospora*, *Penicillium*, *Tolyocladium* (WO 91/00357 published 10 Jan. 1991), and *Aspergillus* hosts such as *A. nidulans* (Ballance et al., *Biochem. Biophys. Res. Commun.*, 112:284-289 (1983); Tilburn et al., *Gene*, 26:205-221 (1983); Yelton et al., *Proc. Natl. Acad. Sci. USA*, 81: 1470-1474 (1984)) and *A. niger* (Kelly and Hynes, *EMBO J.*, 4:475-479 (1985)). Methylotropic yeasts are suitable herein and include, but are not limited to,

yeast capable of growth on methanol selected from the genera consisting of *Hansenula*, *Candida*, *Kloeckera*, *Pichia*, *Saccharomyces*, *Torulopsis*, and *Rhodotorula*. A list of specific species that are exemplary of this class of yeasts may be found in C. Anthony, *The Biochemistry of Methylotrophs*, 269 (1982). Host cells also include insect cells such as *Drosophila* S2 and *Spodoptera* Sf9, as well as plant cells.

Examples of useful mammalian host cell lines include, but are not limited to, HeLa, Chinese hamster ovary (CHO), COS-7, L cells, C127, 3T3, BHK, CHL-1, NSO, HEK293, WI38, BHK, C127 or MDCK cell lines. Another exemplary mammalian cell line is CHL-1. When CHL-1 is used hygromycin is included as a eukaryotic selection marker. CHL-1 cells are derived from RPMI 7032 melanoma cells, a readily available human cell line. Cells suitable for use in this invention are commercially available from the ATCC.

In some embodiments, the host cell is a non-human host cell. In some embodiment, the host cell is a CHO cell. In some embodiments, the host cell is a 293 cell.

The CR2-FH molecules can be isolated by a variety of methods known in the art. In some embodiments, when the CR2-FH molecule is a fusion protein secreted into the growth media, the molecule can be purified directly from the media. If the fusion protein is not secreted, it is isolated from cell lysates. Cell disruption can be done by any conventional method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents. The CR2-FH molecules can be obtained by various methods. These include, but are not limited to, immunoaffinity chromatography, reverse phase chromatography, cation exchange chromatography, anion exchange chromatography, hydrophobic interaction chromatography, gel filtration chromatography, and HPLC. For example, the CR2-FH molecule can be purified by immunoaffinity chromatography using an antibody that recognizes the CR2 portion or an antibody that recognizes the FH portion, or both. In some embodiments, an antibody recognizing the first two N-terminal SCR domains of CR2 is used for purifying the CR2-FH molecule. In some embodiments, the CR2-FH molecule is purified by ion change chromatography.

The peptide may or may not be properly folded when expressed as a fusion protein. These factors determine whether the fusion protein must be denatured and refolded, and if so, whether these procedures are employed before or after cleavage. When denaturing and refolding are needed, typically the peptide is treated with a chaotrope, such as guanidine HCl, and is then treated with a redox buffer, containing, for example, reduced and oxidized dithiothreitol or glutathione at the appropriate ratios, pH, and temperature, such that the peptide is refolded to its native structure.

The CR2-FH molecules described herein may also contain a tag (such as a cleavable tag) for purification. This tag can be fused to the C-terminus or N-terminus of the CR2 portion or the FH portion, and can be used to facilitate protein purification.

In some embodiments, the CR2-FH molecule could be synthesized de novo in whole or in part, using chemical methods well known in the art. For example, the component amino acid sequences can be synthesized by solid phase techniques, cleaved from the resin, and purified by preparative high performance liquid chromatography followed by chemical linkage to form a desired polypeptide. The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing.

The CR2-FH molecules can be assayed for their desired properties using in vitro or in vivo assays. For example, binding of CR2-FH to CR2 ligand can be determined by

surface plasmon resonance method. By way of example, kinetic analysis of the interaction of the CR2-FH with C3dg-biotin can be performed using surface plasmon resonance (SPR) measurements made on a BIAcore 3000 instrument (Biacore AB, Uppsala, Sweden). Human C3dg-biotin can be bound to the surface of BIAcore streptavidin sensor chips by injecting C3dg-biotin over the surface of one flow cell of the chip. Binding can be evaluated over a range of CR2-FH concentrations. Association of CR2-FH molecule with the ligand can be monitored for a certain period of time (such as 120 seconds), after which the complex is allowed to dissociate in the presence of buffer only for an additional period of time (such as 120 seconds). Binding of CR2 fusion protein fragments to C3dg-immobilized flow cells can be corrected for binding to control flow cells. Binding data can be fitted to a 1:1 Langmuir binding model using BIAevaluation Version 3.1 software (BIAcore) and evaluated for best fit. The kinetic dissociation profiles obtained can be used to calculate on and off rates (k_a and k_d) and affinity constants (KD) using the BIAevaluation Version 3.1 program. Other assay methods for ligand binding are known in the art and can also be used.

In vitro zymosan complement assay can be used to determine complement inhibitory activity of CR2-FH molecules. Lysis of rabbit erythrocytes by serum in Mg-EGTA is another measure of activity that can be used. Lysis in Mg-EGTA of human or sheep erythrocytes that have had sialic acid removed provides for additional measures of activity.

Pharmaceutical Compositions

Also provided herein are pharmaceutical compositions comprising a CR2-FH molecule and a pharmaceutically acceptable carrier. The pharmaceutical compositions may be suitable for a variety of modes of administration described herein, including for example systemic or localized administration. The pharmaceutical compositions can be in the form of eye drops, injectable solutions, or in a form suitable for inhalation (either through the mouth or the nose) or oral administration. The pharmaceutical compositions described herein can be packaged in single unit dosages or in multidosage forms.

In some embodiments, the pharmaceutical compositions comprise a CR2-FH molecule and a pharmaceutically acceptable carrier suitable for administration to human. In some embodiments, the pharmaceutical compositions comprise a CR2-FH molecule and a pharmaceutically acceptable carrier suitable for intraocular injection. In some embodiments, the pharmaceutical compositions comprise a CR2-FH molecule and a pharmaceutically acceptable carrier suitable for topical application to the eye. In some embodiments, the pharmaceutical compositions comprise a CR2-FH molecule and a pharmaceutically acceptable carrier suitable for intravenous injection. In some embodiments, the pharmaceutical compositions comprise a CR2-FH molecule and a pharmaceutically acceptable carrier suitable for injection into the arteries (such as renal arteries).

The compositions are generally formulated as sterile, substantially isotonic, and in full compliance with all Good Manufacturing Practice (GMP) regulations of the U.S. Food and Drug Administration. In some embodiments, the composition is free of pathogen. For injection, the pharmaceutical composition can be in the form of liquid solutions, for example in physiologically compatible buffers such as Hank's solution or Ringer's solution. In addition, the CR2-FH pharmaceutical composition can be in a solid form and redissolved or suspended immediately prior to use. Lyophilized compositions are also included.

For oral administration, the pharmaceutical compositions can take the form of, for example, tablets or capsules prepared

by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g., pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulfate). Liquid preparations for oral administration can take the form of, for example, solutions, syrups or suspensions, or they can be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations can be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., ationd oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations can also contain buffer salts, flavoring, coloring and sweetening agents as appropriate.

The present invention in some embodiments provides compositions comprising a CR2-FH molecule and a pharmaceutically acceptable carrier suitable for administration to the eye. Such pharmaceutical carriers can be sterile liquids, such as water and oil, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, and the like. Saline solutions and aqueous dextrose, polyethylene glycol (PEG) and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, sodium stearate, glycerol monostearate, glycerol, propylene, water, and the like. The pharmaceutical composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. The CR2-FH molecule and other components of the composition may be encased in polymers or fibrin glues to provide controlled release of the molecule. These compositions can take the form of solutions, suspensions, emulsions, ointment, gel, or other solid or semisolid compositions, and the like. The compositions typically have a pH in the range of 4.5 to 8.0. The compositions must also be formulated to have osmotic values that are compatible with the aqueous humor of the eye and ophthalmic tissues. Such osmotic values will generally be in the range of from about 200 to about 400 milliosmoles per kilogram of water ("mOsm/kg"), but will preferably be about 300 mOsm/kg.

In some embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for injection intravenously, intraperitoneally, or intravitreally. Typically, compositions for injection are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The compositions may further comprise additional ingredients, for example preservatives, buffers, tonicity agents,

antioxidants and stabilizers, nonionic wetting or clarifying agents, viscosity-increasing agents, and the like.

Suitable preservatives for use in a solution include polyquaternium-1, benzalkonium chloride, thimerosal, chlorobutanol, methyl paraben, propyl paraben, phenylethyl alcohol, edetate disodium, sorbic acid, benzethonium chloride, and the like. Typically (but not necessarily) such preservatives are employed at a level of from 0.001% to 1.0% by weight.

Suitable buffers include boric acid, sodium and potassium bicarbonate, sodium and potassium borates, sodium and potassium carbonate, sodium acetate, sodium biphosphate and the like, in amounts sufficient to maintain the pH at between about pH 6 and pH 8, and preferably, between about pH 7 and pH 7.5.

Suitable tonicity agents are dextran 40, dextran 70, dextrose, glycerin, potassium chloride, propylene glycol, sodium chloride, and the like, such that the sodium chloride equivalent of the ophthalmic solution is in the range 0.9 plus or minus 0.2%.

Suitable antioxidants and stabilizers include sodium bisulfite, sodium metabisulfite, sodium thiosulfite, thiourea and the like. Suitable wetting and clarifying agents include polysorbate 80, polysorbate 20, poloxamer 282 and tyloxapol. Suitable viscosity-increasing agents include dextran 40, dextran 70, gelatin, glycerin, hydroxyethylcellulose, hydroxymethylpropylcellulose, lanolin, methylcellulose, petrolatum, polyethylene glycol, polyvinyl alcohol, polyvinylpyrrolidone, carboxymethylcellulose and the like.

The use of viscosity enhancing agents to provide topical compositions with viscosities greater than the viscosity of simple aqueous solutions may be desirable to increase ocular absorption of the active compounds by the target tissues or increase the retention time in the eye. Such viscosity building agents include, for example, polyvinyl alcohol, polyvinyl pyrrolidone, methyl cellulose, hydroxy propyl methylcellulose, hydroxyethyl cellulose, carboxymethyl cellulose, hydroxy propyl cellulose or other agents known to those skilled in the art. Such agents are typically employed at a level of from 0.01% to 2% by weight.

In some embodiments, there is provided a pharmaceutical composition for delivery of a nucleotide encoding a CR2-FH molecule. The pharmaceutical composition for gene therapy can be in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle or compound is imbedded. Alternatively, where the complete gene delivery system can be produced intact from recombinant cells, e.g., retroviral vectors, the pharmaceutical composition can comprise one or more cells which produce the gene delivery system.

In clinical settings, a gene delivery system for a gene therapeutic can be introduced into a subject by any of a number of methods. For instance, a pharmaceutical composition of the gene delivery system can be introduced systemically, e.g., by intravenous injection, and specific transduction of the protein in the target cells occurs predominantly from specificity of transfection provided by the gene delivery vehicle, cell-type or tissue-type expression due to the transcriptional regulatory sequences controlling expression of the receptor gene, or a combination thereof. In other embodiments, initial delivery of the recombinant gene is more limited with introduction into the animal being quite localized. For example, the gene delivery vehicle can be introduced by catheter, See U.S. Pat. No. 5,328,470, or by stereotactic injection, Chen et al. (1994), Proc. Natl. Acad. Sci., USA 91: 3054-3057. A polynucleotide encoding a CR2-FH molecule can be delivered in a gene

therapy construct by electroporation using techniques described, Dev et al. (1994), *Cancer Treat. Rev.* 20:105-115.

In some embodiments, there is provided a pharmaceutical composition for gene delivery to the eye. Ophthalmic solutions useful for storing and/or delivering expression vectors have been disclosed, for example, in W003077796A2.

Uses of CR2-FH Molecules and Compositions Thereof

The CR2-FH molecules described herein can function to specifically inhibit in vivo complement activation in the alternative complement pathway and inflammatory manifestations that accompany it, such as recruitment and activation of macrophages, neutrophils, platelets, and mast cells, edema, tissue damage, and direct activation of local and endogenous cells. Compositions comprising these molecules can therefore be used for treatment of diseases or conditions that are mediated by excessive or uncontrolled activation of the complement system, particularly diseases or conditions mediated by excessive or uncontrolled activation of the alternative complement pathway. In some embodiments, there are provided methods of treating diseases involving local inflammation process. In some embodiments, there are provided methods of treating diseases associated with FH deficiencies (for example a decrease in FH level, decrease in FH activity, or lack of wild type or protective FH), including, for example, age-related macular degeneration, membranoproliferative glomerulonephritis, proteinuric disease, hemolytic-uremic syndrome, recurrent microbial infection, ischemia reperfusion (such as renal ischemia reperfusion or intestinal ischemia reperfusion), organ transplant rejection, and chronic inflammation such as rheumatoid arthritis.

In some embodiments, there is provided a method of treating a disease in which the alternative complement pathway is implicated (such as macular degeneration, for example AMD) in an individual, comprising administering to the individual an effective amount of a composition comprising a CR2-FH molecule comprising: a) a CR2 portion comprising a CR2 or a fragment thereof, and b) a FH portion comprising a FH or a fragment thereof. In some embodiments, there is provided a method of inhibiting complement activation in an individual having a disease in which the alternative complement pathway is implicated (such as macular degeneration, for example AMD), comprising administering to the individual an effective amount of a composition comprising a CR2-FH molecule comprising: a) a CR2 portion comprising a CR2 or a fragment thereof, and b) a FH portion comprising a FH or a fragment thereof. In some embodiments, there is provided a method of inhibiting inflammation in an individual having a disease in which the alternative pathway is implicated (such as macular degeneration, for example AMD), comprising administering to the individual an effective amount of a composition comprising a CR2-FH molecule comprising: a) a CR2 portion comprising a CR2 or a fragment thereof, and b) a FH portion comprising a FH or a fragment thereof.

“Treating” or “to treat” a disease is defined as administering one or more CR2-FH molecules, with or without other therapeutic agents, in order to palliate, ameliorate, stabilize, reverse, slow, delay, prevent, reduce, or eliminate either the disease or a symptom of the disease, or to retard or stop the progression of the disease or a symptom of the disease. An “effective amount” is an amount sufficient to treat a disease, as defined above.

An “individual” is a vertebrate, preferably a mammal, more preferably a human. Mammals include, but are not limited to, farm animals, sport animals, pets, primates, mice and rats. In some embodiments, the individual is human. In some embodiments, the individual is an individual other than

human. In some embodiments, the individual is an animal model for the study of a disease in which the alternative complement pathway is implicated. Individuals amenable to treatment include those who are presently asymptomatic but who are at risk of developing a symptomatic macular degeneration-related disorder at a later time. For example, human individuals include those having relatives who have experienced such a disease, and those whose risk is determined by analysis of genetic or biochemical markers, by biochemical methods, or by other assays such as T cell proliferation assay. In some embodiments, the individual is a human having a mutation or polymorph in its FH gene that indicates an increased susceptibility to develop a disease in which alternative complement pathway is implicated (such as age-related macular degeneration). In some embodiments, the individual has a wildtype or protective haplotype of FH. Different polymorphs of FH have been disclosed in US Pat. Pub. No. 20070020647, which is incorporated herein in its entirety.

The compositions described herein are particularly useful for treating macular degeneration, such as age-related macular degeneration (AMD). AMD is clinically characterized by progressive loss of central vision which occurs as a result of damage to the photoreceptor cells in an area of the retina called the macula. AMD has been broadly classified into two clinical states: a wet form and a dry form, with the dry form making up to 80-90% of total cases. The dry form is characterized clinically by the presence of macular drusen, which are localized deposits between the retinal pigment epithelium (RPE) and the Bruch’s membrane, and by geographic atrophy characterized by RPE cell death with overlying photoreceptor atrophy. Wet AMD, which accounts for approximately 90% of serious vision loss, is associated with neovascularization in the area of the macula and leakage of these new vessels. The accumulation of blood and fluid can cause retina detachment followed by rapid photoreceptor degeneration and loss of vision. It is generally accepted that the wet form of AMD is preceded by and arises from the dry form.

Analysis of the contents of drusen in AMD patients has shown a large number of inflammatory proteins including amyloid proteins, coagulation factors, and a large number of proteins of the complement pathway. A genetic variation in the complement factor H substantially raises the risk of age-related macular degeneration (AMD), suggesting that uncontrolled complement activation underlies the pathogenesis of AMD. Edward et al., *Science* 2005, 308:421; Haines et al., *Science* 2005, 308:419; Klein et al., *Science* 308:385-389; Hageman et al., *Proc. Natl. Acad. Sci. USA* 2005, 102:7227.

The present invention provides methods of treating AMD (such as wet or dry forms of AMD) by administering an effective amount of a composition comprising a CR2-FH molecule. In some embodiments, the invention provides methods of treating or preventing one or more aspects or symptoms of AMD, including, but not limited to, formation of ocular drusen, inflammation in the eye or eye tissue, loss of photoreceptor cells, loss of vision (including for example visual acuity and visual field), neovascularization (such as choroidal neovascularization or CNV), and retinal detachment. Other related aspects, such as photoreceptor degeneration, RPE degeneration, retinal degeneration, chorioretinal degeneration, cone degeneration, retinal dysfunction, retinal damage in response to light exposure (such as constant light exposure), damage of the Bruch’s membrane, loss of RPE function, loss of integrity of the histoarchitecture of the cells and/or extracellular matrix of the normal macula, loss of function of the cells in the macula, photoreceptor dystrophy,

mucopolysaccharidoses, rod-cone dystrophies, cone-rod dystrophies, anterior and posterior uveitis, and diabetic neuropathy, are also included.

In some embodiments, there are provided methods of treating macular degeneration (such as age-related macular degeneration or AMD) in an individual, comprising administering to the individual an effective amount of a composition comprising a CR2-FH molecule comprising: a) a CR2 portion comprising a CR2 or a fragment thereof, and b) a FH portion comprising a FH or a fragment thereof. In some embodiments, the disease to be treated is a dry form of AMD. In some

embodiments, the disease to be treated is a wet form of AMD. In some embodiments, there are provided methods of treating (such as reducing, delaying, eliminating, or preventing) formation of drusen in the eye of an individual, comprising administering to the individual an effective amount of a composition comprising a CR2-FH molecule comprising: a) a CR2 portion comprising a CR2 or a fragment thereof, and b) a FH portion comprising a FH or a fragment thereof. In some embodiments, there are provided methods of treating (such as reducing, delaying, eliminating, or preventing) inflammation in the eye of an individual, comprising administering to the individual an effective amount of a composition comprising a CR2-FH molecule comprising: a) a CR2 portion comprising a CR2 or a fragment thereof, and b) a FH portion comprising a FH or a fragment thereof. In some embodiments, there are provided methods of treating (such as reducing, delaying, eliminating, or preventing) loss of photoreceptors cells in an individual, comprising administering to the individual an effective amount of a composition comprising a CR2-FH molecule comprising: a) a CR2 portion comprising a CR2 or a fragment thereof, and b) a FH portion comprising a FH or a fragment thereof. In some embodiments, there are provided methods of treating (such as reducing, delaying, eliminating, or preventing) neovascularization associated with AMD, comprising administering to the individual an effective amount of a composition comprising a CR2-FH molecule comprising: a) a CR2 portion comprising a CR2 or a fragment thereof, and b) a FH portion comprising a FH or a fragment thereof. In some embodiments, there are provided methods of treating (such as reducing, delaying, eliminating, or preventing) retinal detachment associated with AMD, comprising administering to the individual an effective amount of a composition comprising a CR2-FH molecule comprising: a) a CR2 portion comprising a CR2 or a fragment thereof, and b) a FH portion comprising a FH or a fragment thereof. In some embodiments, there are provided methods of improving (including for example decreasing, delaying, or blocking loss of) visual acuity or visual field in the eye of an individual, comprising administering to the individual an effective amount of a composition comprising a CR2-FH molecule comprising: a) a CR2 portion comprising a CR2 or a fragment thereof, and b) a FH portion comprising a FH or a fragment thereof.

In addition to macular degeneration, other eye diseases that can be treated by methods of the present invention include, for example, retinitis pigmentosa, diabetic retinopathy, and other eye diseases that involve a local inflammatory process. In some embodiments, the eye disease is diabetic retinopathy. In some embodiments, the eye disease is retinitis pigmentosa.

The methods described herein can also be useful for treatment of certain renal diseases. In some embodiments, there are provided methods of treating membranoproliferative glomerulonephritis type II (MPGN II). MPGN II is a rare kidney disease leading to persisting proteinuria, hematuria, and nephritic syndrome. FH deficiency and dysfunction in MPGN II have been reported in several cases. For example,

mutations in FH have been found in human patients with MPGN II. Pigs of the Norwegian Yorkshire breed have FH defects that are inherited in a recessive pattern. These animals develop MPGN II and show massive complement deposits in the renal glomeruli and die at an early age because of the renal failure. Furthermore, an autoantibody that recognizes FH has been described in a patient with hypocomplementemic MPGN II. Targeting FH to complement activation sites thus will have therapeutic effects on an individual having MPGN II. Accordingly, in some embodiments, there are provided methods of treating MPGN II in an individual, comprising administering to the individual a composition comprising a CR2-FH molecule comprising: a) a CR2 portion comprising a CR2 or a fragment thereof, and b) a FH portion comprising a FH or a fragment thereof. In some embodiments, there are provided methods of treating proteinuria associated with MPGN II. In some embodiments, there are provided methods of treating hematuria associated with MPGN II. In some embodiments, there is provided a method of treating nephritic syndrome associated with MPGN II.

In some embodiments, there are provided methods of treating hemolytic-uremic syndrome (HUS). HUS is a disease consisting of microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure, caused by continuous platelet degradation in the periphery and platelet thrombin in the microcirculation of the kidney. Zipfel, *Seminars in Thrombosis Hemostasis*, 2001, 27(3):191-199. There is now considerable evidence that the nondiarrheal form of HUS (D-HUS) is associated with alternations and mutations of FH. In addition, autoantibodies to FH have been reported in HUS patients. Targeting FH to complement activation sites thus will have therapeutic effects on an individual having HUS. Accordingly, in some embodiments, there are provided methods of treating HUS in an individual, comprising administering to the individual an effective amount of a composition comprising a CR2-FH molecule comprising: a) a CR2 portion comprising a CR2 or a fragment thereof, and b) a FH portion comprising a FH or a fragment thereof. In some embodiments, there are provided methods of treating microangiopathic hemolytic anemia associated with HUS. In some embodiments, there is provided a method of treating thrombocytopenia associated with HUS. In some embodiments, there are provided methods of treating acute renal failure associated with HUS.

In some embodiments, the disease to be treated is systemic lupus erythematosus, such as lupus nephritis. Systemic lupus erythematosus (SLE) is the prototypic autoimmune disease resulting in multiorgan involvement. This anti-self response is characterized by autoantibodies directed against a variety of nuclear and cytoplasmic cellular components. These autoantibodies bind to their respective antigens, forming immune complexes which circulate and eventually deposit in tissues. This immune complex deposition causes chronic inflammation and tissue damage. Complement pathways (including the alternative complement pathway) are implicated in the pathology of SLE, and the methods provided herein are thus useful for treating SLE (such as lupus nephritis).

In some embodiments, the disease to be treated is rheumatoid arthritis. Rheumatoid arthritis is a chronic disease which can exhibit a variety of systemic manifestations. This disease has an unknown etiology and characteristically exhibits a persistent inflammatory synovitis which usually involves peripheral joints in a symmetric distribution. Complement-mediated inflammation which causes cartilage destruction, bone erosions and, ultimately, joint deformities is the most important feature of this disease. Methods provided herein are thus useful for treatment of rheumatoid arthritis.

In some embodiments, the disease to be treated is ischemia reperfusion. Ischemia reperfusion (I/R) injury refers to inflammatory injury to the endothelium and underlying parenchymal tissues following reperfusion of hypoxic tissues. It is a general syndrome that is responsible for both acute and chronic injury to various tissues including, for example, myocardium, central nervous system, hind limb and intestine. Ischemia reperfusion injury can result in necrosis and irreversible cell injury. The complement pathway (including the alternative complement pathway) is a major mediator of I/R injury. Methods provided herein are thus useful for treatment of ischemia reperfusion that occurs in any organ or tissues, including, but not limited to, intestinal ischemia-reperfusion injury, renal ischemia-reperfusion injury, cardiac ischemia-reperfusion injury, ischemia-reperfusion injury of other internal organs such as the lung or liver, central nervous system ischemia-reperfusion injury, ischemia-reperfusion injury of the limbs or digits, trauma-induced hypovolemia, or ischemia-reperfusion injury of any transplanted organ or tissue. Ischemia-reperfusion injury can also occur in conjunction with a variety of other conditions including, but not limited to, stroke, spinal cord injury, trauma-induced hypovolemic shock, and autoimmune diseases such as rheumatoid arthritis (e.g., which can be greatly worsened by ischemic injury of the synovium) or a variety of other inflammatory diseases (diseases mediated by inflammation or wherein inflammation is a symptom that may result in or be associated with ischemic events and reperfusion). Other conditions and diseases in which ischemia-reperfusion injury occurs will be known to those of skill in the art.

In some embodiments, there are provided methods of treating a drusen-associated disease. The term "drusen-associated disease" refers to any disease in which formation of drusen or drusen-like extracellular disease plaque takes place, and for which drusen or drusen-like extracellular disease plaque causes or contributes to thereto or represents a sign thereof. For example, AMD, characterized by the formation of macular drusen, is considered as a drusen-associated disease. Non-ocular drusen-related diseases include, but are not limited to, amyloidosis, elastosis, dense deposit disease, and/or atherosclerosis. The term "drusen-related disease" also includes glomerulonephritis (such as MPGN II).

Other diseases in which the alternative complement pathway is implicated that can be treated by methods of the present invention include, for example: (1) tissue damage due to ischemia-reperfusion following acute myocardial infarction, aneurysm, stroke, hemorrhagic shock, crush injury, multiple organ failure, hypovolemic shock intestinal ischemia, spinal cord injury, and traumatic brain injury; (2) inflammatory disorders, e.g., burns, endotoxemia and septic shock, adult respiratory distress syndrome, cardiopulmonary bypass, hemodialysis; anaphylactic shock, severe asthma, angioedema, Crohn's disease, sickle cell anemia, poststreptococcal glomerulonephritis, membranous nephritis, and pancreatitis; (3) transplant rejection, e.g., hyperacute xenograft rejection; (4) pregnancy related diseases such as recurrent fetal loss and pre-eclampsia, and (5) adverse drug reactions, e.g., drug allergy, IL-2 induced vascular leakage syndrome and radiographic contrast media allergy. Autoimmune disorders including, but not limited to, myasthenia gravis, Alzheimer's disease, multiple sclerosis, emphysema, obesity, rheumatoid arthritis, systemic lupus erythematosus, insulin-dependent diabetes mellitus, acute disseminated encephalomyelitis, Addison's disease, antiphospholipid antibody syndrome, autoimmune hepatitis, Crohn's disease, Goodpasture's syndrome, Graves' disease, Guillain-Barré syndrome, Hashimoto's disease, idiopathic thrombocy-

topenic purpura, pemphigus, Sjögren's syndrome, and Takayasu's arteritis, may also be treated with the inhibitors of the invention.

In some embodiments, the disease to be treated is any of the following: post cardiopulmonary bypass complications; myocardial infarction; ischemia/reperfusion injury; stroke; acute respiratory distress syndrome (ARDS); sepsis; burn injury; inflammation associated with cardiopulmonary bypass and hemodialysis; plasmapheresis; plateletpheresis; leukopheresis; extracorporeal membrane oxygenation (ECMO); heparin-induced extracorporeal LDL precipitation (HELP); radiographic contrast media induced allergic response; transplant rejection; and other inflammatory conditions and autoimmune/immune complex diseases such as multiple sclerosis, myasthenia gravis, pancreatitis, rheumatoid arthritis, Alzheimer's disease, asthma, thermal injury, anaphylactic shock, bowel inflammation, urticaria, angioedema, vasculitis, Sjögren's syndrome, lupus erythematosus, and glomerulonephritis.

The compositions described herein can be administered to an individual via any route, including, but not limited to, intravenous (e.g., by infusion pumps), intraperitoneal, intraocular, intra-arterial, intrapulmonary, oral, inhalation, intravesicular, intramuscular, intra-tracheal, subcutaneous, intrathecal, transdermal, transpleural, topical, inhalational (e.g., as mists of sprays), mucosal (such as via nasal mucosa), gastrointestinal, intraarticular, intracisternal, intraventricular, rectal (i.e., via suppository), vaginal (i.e., via pessary), intracranial, intraurethral, intrahepatic, and intratumoral. In some embodiments, the compositions are administered systemically (for example by intravenous injection). In some embodiments, the compositions are administered locally (for example by intraarterial or intraocular injection).

In some embodiments, the compositions are administered directly to the eye or the eye tissue. In some embodiments, the compositions are administered topically to the eye, for example, in eye drops. In some embodiments, the compositions are administered by injection to the eye (intraocular injection) or to the tissues associated with the eye. The compositions can be administered, for example, by intraocular injection, periocular injection, subretinal injection, intravitreal injection, trans-septal injection, subcleral injection, intrachoroidal injection, intracameral injection, subconjunctival injection, sub-Tenon's injection, retrobulbar injection, peribulbar injection, or posterior juxtasclear delivery. These methods are known in the art. For example, for a description of exemplary periocular routes for retinal drug delivery, see Periocular routes for retinal drug delivery, Raghava et al. (2004), *Expert Opin. Drug Deliv.* 1(1):99-114. The compositions may be administered, for example, to the vitreous, aqueous humor, sclera, conjunctiva, the area between the sclera and conjunctiva, the retina choroids tissues, macula, or other area in or proximate to the eye of an individual. The compositions can also be administered to the individual as an implant. Preferred implants are biocompatible and/or biodegradable sustained release formulations which gradually release the compounds over a period of time. Ocular implants for drug delivery are well-known in the art. See, e.g., U.S. Pat. Nos. 5,501,856, 5,476,511, and 6,331,313. The compositions can also be administered to the individual using iontophoresis, including, but are not limited to, the ionophoretic methods described in U.S. Pat. No. 4,454,151 and U.S. Pat. App. Pub. No. 2003/0181531 and 2004/0058313.

In some embodiments, the compositions are administered intravascularly, such as intravenously (IV) or intraarterially. In some embodiments (for example for the treatment of renal

diseases), the compositions are administered directly into arteries (such as renal arteries).

The optimal effective amount of the compositions can be determined empirically and will depend on the type and severity of the disease, route of administration, disease progression and health, mass and body area of the individual. Such determinations are within the skill of one in the art. The effective amount can also be determined based on in vitro complement activation assays. Examples of dosages of CR2-FH molecules which can be used for methods described herein include, but are not limited to, an effective amount within the dosage range of any of about 0.01 µg/kg to about 300 mg/kg, or within about 0.1 µg/kg to about 40 mg/kg, or with about 1 µg/kg to about 20 mg/kg, or within about 1 µg/kg to about 10 mg/kg. For example, when administered intraocularly, the composition may be administered at low microgram ranges, including for example about 0.1 µg/kg or less, about 0.05 µg/kg or less, or 0.01 µg/kg or less. In some embodiments, the amount of CR2-FH administered to an individual is about 10 µg to about 500 mg per dose, including for example any of about 10 µg to about 50 µg, about 50 µg to about 100 µg, about 100 µg to about 200 µg, about 200 µg to about 300 µg, about 300 µg to about 500 µg, about 500 µg to about 1 mg, about 1 mg to about 10 mg, about 10 mg to about 50 mg, about 50 mg to about 100 mg, about 100 mg to about 200 mg, about 200 mg to about 300 mg, about 300 mg to about 400 mg, or about 400 mg to about 500 mg per dose.

The CR2-FH compositions may be administered in a single daily dose, or the total daily dose may be administered in divided dosages of two, three, or four times daily. The compositions can also be administered less frequently than daily, for example, six times a week, five times a week, four times a week, three times a week, twice a week, once a week, once every two weeks, once every three weeks, once a month, once every two months, once every three months, or once every six months. The compositions may also be administered in a sustained release formulation, such as in an implant which gradually releases the composition for use over a period of time, and which allows for the composition to be administered less frequently, such as once a month, once every 2-6 months, once every year, or even a single administration. The sustained release devices (such as pellets, nanoparticles, microparticles, nanospheres, microspheres, and the like) may be administered by injection or surgical implantation in various locations in the eye or tissue associated with the eye, such as intraocular, intravitreal, subretinal, periocular, subconjunctival, or sub-Tenons.

The pharmaceutical compositions can be administered alone or in combination with other molecules known to have a beneficial effect on retinal attachment or damaged retinal tissue, including molecules capable of tissue repair and regeneration and/or inhibiting inflammation. Examples of useful cofactors include anti-VEGF agents (such as an antibody against VEGF), basic fibroblast growth factor (bFGF), ciliary neurotrophic factor (CNTF), axokine (a mutein of CNTF), leukemia inhibitory factor (LIF), neurotrophin 3 (NT-3), neurotrophin-4 (NT-4), nerve growth factor (NGF), insulin-like growth factor II, prostaglandin E2, 30 kD survival factor, taurine, and vitamin A. Other useful cofactors include symptom-alleviating cofactors, including antiseptics, antibiotics, antiviral and antifungal agents, and analgesics and anesthetics.

Gene Therapy

The CR2-FH molecules can also be delivered by expression of the CR2-FH fusion protein in vivo, which is often referred to as "gene therapy". For example, cells may be engineered with a polynucleotide (DNA or RNA) encoding

for the fusion protein ex vivo, the engineered cells are then provided to an individual to be treated with the fusion protein. Such methods are well-known in the art. For example, cells may be engineered by procedures known in the art by use of a retroviral particle containing RNA encoding for the fusion protein of the present invention.

Local delivery of the fusion proteins of the present invention using gene therapy may provide the therapeutic agent to the target area, for example to the eye or the eye tissue.

Methods of gene delivery are known in the art. These methods include, but are not limited to, direct DNA transfer, see, e.g., Wolff et al. (1990) *Science* 247: 1465-1468; 2) Liposome-mediated DNA transfer, see, e.g., Caplen et al. (1995) *Nature Med.* 3:39-46; Crystal (1995) *Nature Med.* 1:15-17; Gao and Huang (1991) *Biochem. Biophys. Res. Comm.* 179:280-285; 3) Retrovirus-mediated DNA transfer, see, e.g., Kay et al. (1993) *Science* 262:117-119; Anderson (1992) *Science* 256:808-813; 4) DNA Virus-mediated DNA transfer. Such DNA viruses include adenoviruses (preferably Ad2 or Ad5 based vectors), herpes viruses (preferably herpes simplex virus based vectors), and parvoviruses (preferably "defective" or non-autonomous parvovirus based vectors, more preferably adeno-associated virus based vectors, most preferably AAV-2 based vectors). See, e.g., Ali et al. (1994) *Gene Therapy* 1:367-384; U.S. Pat. No. 4,797,368, incorporated herein by reference, and U.S. Pat. No. 5,139,941.

Retroviruses from which the retroviral plasmid vectors hereinabove mentioned may be derived include, but are not limited to, Moloney Mouse Leukemia Virus, spleen necrosis virus, retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, adenovirus, Myeloproliferative Sarcoma Virus, and mammary tumor virus. In one embodiment, the retroviral plasmid vector is derived from Moloney Mouse Leukemia Virus.

Adenoviruses have the advantage that they have a broad host range, can infect quiescent or terminally differentiated cells, such as neurons or hepatocytes, and appear essentially non-oncogenic. See, e.g., Ali et al. (1994), supra, p. 367. Adenoviruses do not appear to integrate into the host genome. Because they exist extrachromosomally, the risk of insertional mutagenesis is greatly reduced. Ali et al. (1994), supra, p. 373.

Adeno-associated viruses exhibit similar advantages as adenoviral-based vectors. However, AAVs exhibit site-specific integration on human chromosome 19 (Ali et al. (1994), supra, p. 377).

The gene therapy vectors include one or more promoters. In some embodiments, the vector has a promoter that drives expression in multiple cell types. In some embodiments, the vector has a promoter that drives expression in specific cell types (such as cells of retina or cells in the kidney). Suitable promoters which may be employed include, but are not limited to, the retroviral LTR; the SV40 promoter; and the human cytomegalovirus (CVM) promoter described in Miller et al. (1989) *Biotechniques* 7(9):980-990, or any other promoter (e.g., cellular promoters such as eukaryotic cellular promoters including, but not limited to, the histone, pol III, and .beta.-actin promoters). Other viral promoters which may be employed include, but are not limited to, adenovirus promoters, thymidine kinase (TK) promoters, and B19 parvovirus promoters. The selection of a suitable promoter will be apparent to those skilled in the art from the teachings contained herein.

The nucleic acid sequence encoding a CR2-FH fusion protein is under the control of a suitable promoter. Suitable promoters which may be employed include, but are not lim-

ited to, adenoviral promoters, such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the respiratory syncytial virus (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; the ApoA1 promoter; human globin promoters; viral thymidine kinase promoters, such as the Herpes Simplex thymidine kinase promoter; retroviral LTRs (including the modified retroviral LTRs hereinabove described); the β -actin promoter; and human growth hormone promoter.

Retroviral plasmid vectors can be employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which maybe transfected are described in Miller (1990) *Human Gene Therapy* 1:5-14. The vectors may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and CaPO₄ precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host. The producer cell line generates infectious retroviral vector particles which include the nucleic acid sequence(s) encoding the polypeptides. Such retroviral vector particles then may be employed, to transduce eukaryotic cells, either in vitro or in vivo. The transduced eukaryotic cells will express the nucleic acid sequence(s) encoding the polypeptide. Eukaryotic cells which may be transduced include, but are not limited to, embryonic stem cells, embryonic carcinoma cells, as well as hematopoietic stem cells, hepatocytes, fibroblasts, myoblasts, keratinocytes, endothelial cells, and bronchial epithelial cells.

In some embodiments, gene delivery vectors which direct expression of CR2-FH in the eye are used. Vectors for gene delivery to the eye are known in the art, and have been disclosed, for example, in U.S. Pat. No. 6,943,153, and U.S. Patent Application Publication Nos. US20020194630, US20030129164, and US200600627165.

In some embodiments, the complement activation is inhibited by contacting a body fluid with a composition comprising a CR2-FH molecule ex vivo under conditions that permit the CR2-FH molecule to function to inhibit complement activation. Suitable body fluids include those that can be returned to the individual, such as blood, plasma, or lymph. Affinity adsorption apheresis is described generally in Nilsson et al. (1988) *Blood* 58(1):38-44; Christie et al. (1993) *Transfusion* 33:234-242; Richter et al. (1997) *ASAIO J.* 43(1):53-59; Suzuki et al. (1994) *Autoimmunity* 19: 105-112; U.S. Pat. No. 5,733,254; Richter et al. (1993) *Metabol. Clin. Exp.* 42:888-894; and Wallukat et al. (1996) *Int'l J. Card.* 54:1910195.

Accordingly, the invention include methods of treating one or more diseases described herein in an individual comprising treating the individual's blood extracorporeally (i.e., outside the body or ex vivo) with a composition comprising a CR2-FH molecule under conditions that permit the molecule to function to inhibit complement activation, and returning the blood to the individual.

Unit Dosages, Articles of Manufacture, and Kits

Also provided are unit dosage forms of CR2-FH molecule compositions, each dosage containing from about 0.01 mg to about 50 mg, including for example any of about 0.1 mg to about 50 mg, about 1 mg to about 50 mg, about 5 mg to about 40 mg, about 10 mg to about 20 mg, or about 15 mg of the CR2-FH molecule. In some embodiments, the unit dosage forms of CR2-FH molecule composition comprises about any of 0.01 mg-0.1 mg, 0.1 mg-0.2 mg, 0.2 mg-0.25 mg, 0.25 mg-0.3 mg, 0.3 mg-0.35 mg, 0.35 mg-0.4 mg, 0.4 mg-0.5 mg, 0.5 mg-1.0 mg, 10 mg-20 mg, 20 mg-50 mg, 50 mg-80 mg, 80

mg-100 mg, 100 mg-150 mg, 150 mg-200 mg, 200 mg-250 mg, 250 mg-300 mg, 300 mg-400 mg, or 400 mg-500 mg CR2-FH molecule. In some embodiments, the unit dosage form comprises about 0.25 mg CH2-FH molecule. The term "unit dosage form" refers to a physically discrete unit suitable as unitary dosages for an individual, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical carrier, diluent, or excipient. These unit dosage forms can be stored in suitable packaging in single or multiple unit dosages and may also be further sterilized and sealed.

Also provided are articles of manufacture comprising the compositions described herein in suitable packaging. Suitable packaging for compositions (such as ophthalmic compositions) described herein are known in the art, and include, for example, vials (such as sealed vials), vessels, ampules, bottles, jars, flexible packaging (e.g., sealed Mylar or plastic bags), and the like. These articles of manufacture may further be sterilized and/or sealed.

The present invention also provides kits comprising compositions (or unit dosages forms and/or articles of manufacture) described herein and may further comprise instruction(s) on methods of using the composition, such as uses described herein. The kits described herein may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for performing any methods described herein.

EXAMPLES

Example 1

Exemplary Sequences of CR2-FH Molecules and Signal Peptides

FIGS. 4-6 provide exemplary amino acid sequences of CR2-FH molecules described herein (SEQ ID NOs: 5-10). "nnn" represents an optional linker.

FIG. 7 provides exemplary amino acid sequences of signaling peptides described herein (SEQ ID NOs: 11 and 13) and polynucleotides encoding the signaling peptides (SEQ ID NOs:12 and 14).

FIG. 9 provides amino acid sequence of a mouse CR2-FH fusion protein (designated as CR2-fH or CR2NLFH) (SEQ ID NO:17) and a polynucleotide that encodes a mouse CR2-FH plus the signal peptide (SEQ ID NO:18).

FIG. 10 provides the DNA sequence of CR2NLFHFH, a mouse CR2-FH fusion protein containing a CR2 portion and two FH portions without a linker sequence (SEQ ID NO:19).

FIG. 11 provides the DNA sequence of CR2LFHFH, a mouse CR2-FH fusion protein containing a CR2 portion linked to two FH portions via a linker sequence (SEQ ID NO:20).

FIG. 20 provides amino acid sequence of a human CR2-FH fusion protein (designated as human CR2-fH or CR2fH) (SEQ ID NO:21) and a polynucleotide that encodes a human CR2-fH plus the signal peptide (SEQ ID NO:22).

FIG. 21 provides amino acid sequence of a human CR2-FH fusion protein containing two FH portions (designated as human CR2-FH2 or CR2fH2 or human CR2fH2) (SEQ ID NO:23) and a polynucleotide that encodes a human CR2-FH2 plus the signal peptide (SEQ ID NO:24).

In Vitro Inhibition of Alternative Pathway by
CR2-FH

Mouse fusion proteins containing the first four SCR domains of CR2 and the first five SCR domains of FH (with linker (CR2LFH) or without linker (CR2NLFH or CR2-fH)) were made by recombinant DNA cloning and gene expression method. The sequence for one of the CR2-FH fusion proteins is provided in FIG. 9. SEQ ID NO:17 is the polypeptide sequence of the CR2-FH fusion protein. SEQ ID NO:18 is the nucleotide used to encode the fusion protein, as well as a signal peptide at the N-terminus of the signal peptide.

A mouse CR2-FH fusion protein (designated as CR2LFHFH, CR2-fH2 or CR2-fHH) containing the first four SCR domains of CR2 and two tandemly linked FH portions (each containing the first five SCR domains of FH) was also made. The CR2 portion and the first FH portion was linked by a linker sequence. The DNA sequence (including the DNA encoding the signal peptide) of CR2LFHFH is provided in FIG. 11 (SEQ ID NO:20).

In vitro assays for activation of the alternative pathway were conducted as essentially described in Quigg et al., *J. Immunol.* 1998, 160(9):4553-60. Factor H (fH) or CR2-Crry were used as controls in the experiment. Specifically, 50 mg of zymosan beads in 10 ml of 0.15M NaCl were activated by boiling for 60 minutes, and washed twice in PBS. In each reaction mixture add: 1) 10 mM EGTA and 5 mM MgCl₂ (final concentration); 2) 1×10⁷ beads; 3) 10 mM EDTA (negative control 1) or HIC serum (negative control 2) or increasing concentration of one of the CR2-FH fusion proteins or control proteins; 4) 10 μl of serum; and 5) PBS to bring the total volume to 100 μl. The mixtures were incubated at 37° C. for 20 minutes, and the reactions were stopped by addition of 10 mM EDTA (final concentration). The beads were washed twice with cold PBSB (PBS with 1% BSA), and incubated with FTIC-conjugated goat-anti-C3 antibody for one hour on ice. The sample was then washed twice in PBSB, resuspended with 1% paraformaldehyde and analyzed under flow cytometry.

FIG. 12A provides a graphic representation of data obtained in an in vitro zymosan complement assay using mouse CR2-FH fusion proteins (CR2-fH) and factor H alone (fH). As shown in the figure, CR2-fH was significantly more effective than FH in inhibiting complement activation. FIG. 12B provides a graphic representation of data obtained in an in vitro zymosan complement assay using the first five SCR domains of mouse FH (FH 15) and the first four domains of mouse CR2 (CR2). The first five SCR domains of mouse FH had an EC₅₀ of 250 nM, which approximately equal to the amount of FH in serum. The molecule having the first four domains of CR2 has no inhibitory effect at all. These data demonstrate that the effect seen with CR2-FH is due to the combined effects of the two portions of the molecule, rather than the independent function of each portion.

FIG. 13 provides a graphic representation of data obtained in an in vitro zymosan complement assay using mouse CR2-FH fusion protein with linker (CR2LFH), CR2-FH fusion protein without linker (CR2NLFH), CR2-FH-FH with linker (CR2LFHFH), and CR2-Crry. As shown in the figure, CR2-FH was more effective than CR2-Crry in inhibiting complement activation of the alternative pathway. CR2LFH and CR2NLFH were equally effective in inhibiting complement

activation of the alternative pathway. CR2LFHFH is much more effective than CR2LFH and CR2NLFH.

Example 3

Treatment of Intestinal Ischemia and Reperfusion
Injury by CR2-FH

This experiment shows treatment of intestinal ischemia and reperfusion injury in a mouse model.

Intestinal Ischemia Reperfusion Injury. Three adult male mice aged 8 weeks and weighing 20-25 g were anesthetized with 10 mg/kg ketamine and 6 mg/kg xylazine by i.p. injection. Animals were breathing spontaneously and body temperature was maintained using a heat mat for the entire experiment. A medial laparotomy was performed and the intestines were carefully moved allowing access to the superior mesenteric artery. The superior mesenteric artery was clamped using a microsurgical clamp (Fine Instruments, USA). Ischemia was confirmed by palor of the small intestine. Sham treated mice underwent laparotomy without clamping of superior mesenteric artery. After 30 min. ischemia the arterial clamp was removed allowing reperfusion of the mesenteric vasculature. Animals were sutured using 6.0 ethicon suture and allowed to reperfuse for 2 hours. 0.1 mg or 0.05 mg CR2-fH, or control (PBS) were administered i.v. 30 minutes post reperfusion and animals were sacrificed 90 minutes later following a total of 2 hours of reperfusion.

Histology. Tissue samples for histological staining were taken from the intestine and either fixed in 10% formalin at 4° C. overnight and subsequently processed to paraffin, or frozen in liquid nitrogen for immunofluorescence analysis. Sections of intestine from each animal were stained with hematoxylin and eosin and scored for mucosal damage and villi height as previously described (46). Briefly, a score of 0 was assigned to a normal villus; villi with tip distortion were scored as 1; villi lacking goblet cells and containing Gugenheims' spaces were scored 2; villi with patchy disruption of the epithelial cells were scored 3; villi exposed but intact lamina propria and epithelial cell sloughing were assigned 4; villi in which lamina propria were exuding were scored as 5, and finally, villi displaying hemorrhage or denuded villi were scored as 6. All histological evaluations were carried out in a blinded fashion.

The results of the experiment are shown in FIG. 14A. As shown in the figure, both 0.1 mg and 0.05 mg of CR2-fH showed protective effect in the animal model compared to the control animals even though the control animals had normal levels of circulating endogenous factor H (about 0.5 mg/ml) in excess of the amounts of CR2-fH administered.

Example 3.1

Treatment of Intestinal Ischemia and Reperfusion
Injury by Mouse CR2-FH

The experiment was carried out essentially as disclosed in Example 3.

Briefly, 0.05 mg, 0.1 mg, or 0.2 mg of mouse CR2-fH or mouse CR2-fH2 (CR2-fHH) were administered i.v. 30 minutes post reperfusion and animals were sacrificed 90 minutes later for histology analysis. The results of the experiment are shown in FIG. 14B. As shown in FIG. 14B, both mouse CR2-fH and mouse CR2-fHH protected the intestine from complement-mediated ischemia reperfusion injury.

Example 3.2

Treatment of Intestinal Ischemia and Reperfusion Injury by Mouse CR2-FH

This experiment shows the effects of mouse CR2-fH and CR2-fH2 on alternative complement pathway and intestinal ischemia reperfusion. The experiments are carried out essentially as described above.

In vitro assays demonstrated that mouse CR2-fH was significantly more effective in inhibiting the alternative pathway of complement than CR2-Crry, and that mouse CR2-fH2 was about 2-fold more effective than mouse CR2-fH. The complement inhibitory activity of mouse CR2-fH was dependent on CR2-mediated targeting as demonstrated by anti-CR2 antibody blocking experiments. Furthermore, purified mouse factor H had only minimal complement inhibitory activities in the in vitro assays.

Mouse CR2-fH and mouse CR2-fH2 targeted to sites of local and remote (lung) complement activation following intestinal ischemia and reperfusion injury, and both proteins protected the intestinal mucosa and the lung parenchyma from injury at a low dose and in a dose dependent manner. Although mouse CR2-fH2 was a more potent inhibitor of the alternative complement pathway than mouse CR2-fH in vitro, there was no difference in the protective effect of the two proteins in the in vivo model. Compared to CR2-Crry, an approximate 2-fold higher dose of mouse CR2-fH was required to provide equivalent protection from local injury.

Example 4

Treatment of Renal Ischemia Reperfusion by Mouse CR2-FH

This example shows the effect of CR2-FH on renal ischemia reperfusion.

Protocol for Induction of Ischemic ARF. Mice weighing 20-25 grams were anesthetized with 300 μ l of 2,2,2-Tribromoethanol (Sigma-Aldrich) injected intra-peritoneally. After the mice were anesthetized, they were placed on a heating pad to maintain their body temperature during surgery. Laparotomies were then performed, and the renal pedicles were located and isolated by blunt dissection. The pedicles were clamped with surgical clips (Miltex Instrument Company, Inc.), and occlusion of blood flow was confirmed by visual inspection of the kidneys. The clamps were left in place for 24 minutes and then released. The time of ischemia was chosen to obtain a reversible model of ischemic ARF with a minimum of vascular thrombosis, and to avoid animal mortality. The kidneys were observed for approximately one minute to ensure blood re-flow. After 15 minutes of reperfusion the mice received 0.25 mg of the mouse CR2-fH (CR2NLFH) intraperitoneally. Fascia and skin were sutured with 4-0 silk (United States Surgical). The mice were volume resuscitated with 0.5 ml of normal saline and kept in an incubator at 29° C. to maintain body temperature.

After 24 hours of reperfusion the mice were anesthetized, and blood was obtained by cardiac puncture. Laparotomy was performed and the kidneys were harvested. The study protocol was approved by the University of Colorado Health Sciences Center Animal Care and Use Committee.

Serum Urea Nitrogen Measurements. Serum urea nitrogen was determined for each mouse using a Beckman Autoana-

lyzer (Beckman). The result of is shown in FIG. 15A. As shown in the figure, serum urea nitrogen was reduced in mouse CR2-fH treated animals, indicating preservation of kidney function.

5 Renal Morphology. After the kidneys were removed from the mice, sagittal sections were fixed in 4% paraformaldehyde. After being embedded in paraffin, four μ m sections were cut and stained with periodic acid Schiff. The sections were evaluated by a renal pathologist in a blinded fashion. 10 The cortex and outer stripe of the outer medulla were assessed for epithelial necrosis, loss of brush border, tubular dilatation and cast formation. At least ten fields (400 \times) were reviewed for each slide, and the percentage of tubules displaying these findings was determined. The kidney sections were scored as follows based on the percentage of affected tubules: 0, none; 15 1, <10%, 2, 11-25%, 3, 26-45%, 4, 46-75%, 5, >75%. The result of the experiment is shown in FIG. 15B. As shown in the figure, CR2-fH showed protective effect in the animal model compared to the control animal.

20 Immunofluorescence. For immunofluorescence, sagittal sections of the kidneys were snap frozen in OCT compound (Sakura Finetek). Four μ m sections were cut with a cryostat and stored at -70° C. The slides were later fixed with acetone and incubated with the FITC conjugated antibody to mouse 25 C3 (Cappel). After hybridization with the antibody for one hour at room temperature, the slides were counterstained with hematoxylin (Vector Laboratories, Inc.). The results of the experiment are shown in FIGS. 15C and 15D. As shown in the figure, more C3 was deposited into kidneys of sham treated mice (15C) relative to mouse CR2-fH-treated mice (15D).

Example 5

Treatment of Age-Related Macular Degeneration by CR2-FH

35 Constant light exposed albino rats are used as animal models for age-related macular degeneration (dry AMD). Five to eight animals are injected intraocularly under anesthesia every other day with a CR2-FH fusion protein (1 μ l of 4.3 mg/ml stock solution), starting with the first injection the day prior to the onset of continuous light exposure (days -1, 1, 3, 40 5, 7). One eye serves as the experimental, while the other eye serves as the PBS-injected control eye. Animals are tested with ERG on day 8 and then euthanized for histology and PCR analysis. Number of rows of photoreceptors in eyes injected with CR2-FH are compared with those of the PBS control eyes. 45 50

The effect of CR2-FH are measured using three parameters: functional activity (ERG and DC potentials, i.e., photoreceptor and RPE responses), histology and measures of inflammation (e.g., gene expression by RT-PCR and protein expression by immunohistochemistry). 55

In a second animal model (wet AMD), we test whether eliminating complement activators reduces choroidal neovascularization (CNV). CNV is produced in five to eight rats with a Krypton laser (200 mW, 50 μ m, 0.05 sec) and documented in choroidal flatmounts after fluorescein injections. 60

The effect of CR2-FH are measured using four parameters: functional activity (ERG and DC potentials, i.e., photoreceptor and RPE responses), histology, vascular integrity (choroidal flatmounts after fluorescein injections) and measures of inflammation (e.g., gene expression by RT-PCR and protein expression by immunohistochemistry). 65

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Example 6

Reduction in CNV Volume by Mouse CR2-FH

For generation of CNV, 3-month-old animals were anesthetized using xylazine and ketamine (20 and 80 mg/kg, respectively) and pupils dilated with a drop of phenylephrine HCl (2.5%) and atropine sulfate (1%). Argon laser photocoagulation (532 nm, 50 μ m spot size, 0.05 s duration, 250 mW) was used to generate four laser spots in each eye surrounding the optic nerve, using a handheld coverslip as a contact lens. A bubble formed at a laser spot indicated the rupture of Bruch's membrane. Nozaki et al., *Proc. Natl. Acad. Sci.* 2006, 103(7):2328-33.

For assessment of CNV lesions, CNV size was determined in flat-mount preparations of RPE/choroids stained with isolectin B (which binds to terminal β -D-galactose residues on the surface of endothelial cells and selectively labels the mouse vasculature). Fluorescence measurements taken in 2 μ m sections using confocal microscopy were used for size determination. In short, a Z-stack of images through the CNV lesion was obtained, using the same laser intensity setting for all experiments. For each slice the overall fluorescence was determined and plotted against depth.

For electroretinography, animals were anesthetized using xylazine (20 mg/kg bodyweight) and ketamine (80 mg/kg bodyweight). Pupils were dilated with a drop of phenylephrine HCl (2.5%) and tropicamide (1%). Body temperature was stabilized via a DC-powered heating pad held at 37° C. The ERG setup used was previously described by Rohrer et al., *J. Neurosci.*, 1999, 19(20): 8919-30 and was built according to Lyubarsky et al., *J. Neurosci.*, 1996, 16(2):563-571. Stimulus light intensity was controlled using neutral density filters. Stimulus paradigms: Animals were dark-adapted overnight and ERGs will be recorded. Rods were analyzed in response to single-flash stimuli of increasing light intensity. The single-flash responses were an average of at least 3 flashes with an inter-stimulus interval (ISI) of 15 s to 2 min (lowest intensity to highest, respectively). The different ISIs ensured that ERG amplitudes at a given intensity were identical between the first and the last flash. Data analysis: For all ERG recordings, a-wave amplitude were measured from baseline to trough; b-wave amplitude were measured from a-wave trough or baseline to peak of b-wave, and implicit times were measured from onset of stimulus to a-wave trough or b-wave peak.

In one experiment, mice were treated with intravenous mouse CR2-fH (250 μ g) 30 minutes post laser burn, 48 hours post laser burn, and 6 hours post laser burn. 6 days post laser burn, retinal function was assessed, then mice were sacrificed for histology.

FIG. 16 shows a- and b-wave retinal responses in mice treated with or without CR2-fH. As shown in FIG. 16, both a- and b-waves of retinal response were protected by CR2-fH treatment relative to PBS treatment. FIGS. 17A and 17B show isolectin-b staining of lesions 6 days post laser burn. FIG. 17C shows quantification of lesion sizes based on the isolectin-b staining. As shown in FIGS. 17A-C, mice treated with CR2-fH show significant reduction in lesion size as compared to animals treated with PBS.

In a separate experiment, 1 μ g mouse CR2-fH was administered intraoptically immediately after laser burn, 48 hours post burn, and 96 hours post burn. Eyes were collected at day 6 for histology. Lesions were visualized by isolectin-b staining. The results are shown in FIG. 18. FIGS. 18A and 18B show isolectin-b staining of lesions 6 days post laser burn. FIG. 18C shows quantification of lesion size based on the

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isolectin-b staining. As shown in FIGS. 18A-C, CR2-fH delivered directly to the eye reduces spread of the lesion.

Example 7

Delay of Onset of Antibody-Mediated Rejection in a Mouse Heterotopic Heart Transplant Model by Mouse CR2-FH

In this experiment, hearts were heterotopically transplanted from C3H donor mice into Balb/c recipient mice. This strain combination promotes a TH2 immune phenotype which promotes acute vascular rejection, and is characterized by anti-graft antibody production and graft deposition of complement activation fragments.

Recipient mice were treated with 1) PBS, i.v., 2) a single 0.25 mg dose of mouse CR2-fH, i.v. 30 minutes post reperfusion, and 3) multiple doses of 0.25 mg mouse CR2-fH i.v. starting 30 minutes post reperfusion and then every three days thereafter.

Hearts were harvested 24 hours post reperfusion for analysis. Mouse CR2-fH treated animals were protected from ischemia and reperfusion injury as assessed by histology, the absence of C3, a reduction in neutrophil infiltration, and a reduction in inflammatory cytokines.

The effects of mouse CR2-fH on acute vascular rejection are shown in FIG. 21. As shown in the figure, control heart transplant recipients survived 7.1 ± 1 days, compared to 11.1 ± 1.6 days (single dose group) and 10.7 ± 1.3 days (multiple dose group). There is a significant improvement in survival in mice treated with mouse CR2-fH when compared to controls ($p=0.02$).

At the time of harvest there were no obvious differences in pathological rejection profiles or in the levels of anti-donor antibodies between any of the groups. Interestingly, there appears to be no significant improvement in survival associated with the administration of multiple doses of mouse CR2-fH when compared to the single dose group ($p<0.05$).

Example 8

Inhibition of alternative complement pathway by human CR2-FH

The protein sequences of human CR2-FH (SEQ ID NO:21, also designated as CR2fH) and human CR2-FH2 (SEQ ID NO:23, also designated as CR2fH2), not including signal peptides, are shown in FIGS. 20 and 21, respectively. The nucleic acid sequences of human CR2-FH (SEQ ID NO: 22) and human CR2-FH2 (SEQ ID NO:24), including nucleotide sequences for signal peptides, are shown in FIGS. 20 and 21, respectively.

Human CR2-FH and human CR2-FH2 were purified from transfected 293 cell supernatants by affinity chromatography using HB5-sepharose, which contains anti-human CR2 monoclonal antibody HB5 (ATCC catalog # HB-135) linked to CNBr-activated sepharose (Amersham Biosciences). Crude CR2-FH or CR2-FH2 supernatants were passed over the matrix, washed with PBS, and eluted in 0.1M glycine-HCl, pH 3.0. The eluted fraction was immediately neutralized by the addition of 1M Tris-Cl, pH 9.0 followed by exchange into PBS using centricon columns (Millipore). 300 ng of nonreduced, purified CR2-FH and CR2-FH2 were resolved on SDS-PAGE and visualized by Commassie staining. CR2-FH

was present as two distinct proteins, as determined by mass spectrometry (Alphalyse, Palo Alto, Calif.) of 64.0 and 65.3 kDa which resolved into a single band following deglycosylation, while CR2-FH2 was a single species of 99.2 kDa. The inherent secondary structure of these molecules makes them run smaller than their actual molecular weight under non-reducing conditions.

The effects of human CR2-FH and human CR2-FH2 on alternative pathway specific C3b deposition onto zymosan particles are shown in FIG. 22A. Briefly, Zymosan particles were incubated in PBS containing 5 mM Mg²⁺, 10 mM EGTA, 10% human serum, and increasing concentrations of CR2-FH and CR2-FH2 for 30 minutes at room temperature with FITC conjugated goat anti-human C3 antibody. Zymosan was pelleted and washed, followed by FACS analysis. As shown in FIG. 24A, both CR2-FH and CR2-FH2 inhibited activation of the alternative complement pathway. Similar results were obtained by incubating with mouse serum followed by detection with FITC conjugated goat anti-mouse C3 antibody. Significantly, there was 200-400 nM FH present in the assay system. The CR2-FH had an EC₅₀ of 8-22 nM, which was 20-fold lower than the amount of FH present in the assay, demonstrating a clear benefit of targeted FH over endogenous FH.

The effects of human CR2-FH and human CR2-FH2 on alternative pathway-mediated erythrocyte lysis are shown in FIG. 22B. Briefly, rabbit erythrocytes (1×10⁸) were incubated with varying concentrations of CR2-FH or CR2-FH2 in 1×GVB++(Boston BioProducts) and 17% human serum for 30 minutes at 37° C. The reaction was stopped with the addition of one tenth volume cold PBS followed by centrifugation to pellet unlysed erythrocytes. Hemolysis was quantified by measuring OD_{415nm}. As shown in FIG. 24B, both CR2-FH and CR2-FH2 significantly inhibited activation of the alternative complement pathway. Significantly, there was 340-680 nM FH present in the assay. The CR2-FH had an EC₅₀ of 20-30 nM, which was 15-20 fold lower than the amount of FH present in the assay, demonstrating a clear benefit of targeted FH over endogenous FH.

Inhibition of the Alternative Complement Pathway by Mouse CR2-FH

This example shows inhibition of the alternative complement pathway by mouse CR2-FH using serum for mice deficient in the classical pathway.

ELISA assay with immune complexes of collagen-anti-collagen antibodies on the plates were used. C3 deposition/activation was measured by using anti-C3b antibody in the presence of serum from wildtype or from C4⁻/C4⁻ mice. Different amounts of full length mouse FH (2 μg/10 μl), the first four SCR domains of mouse CR2 (2 μg/10 μl), and mouse CR2-FH (2 μg/10 μl) were added to the serum. The result of the in vitro study is shown in FIG. 23. As shown in the figure, mouse CR2-FH had little effect on C3b deposition using serum from wildtype mice. By contrast, mouse CR2-FH almost completely prevented C3b deposition in serum from classical pathway deficient mice. Mouse FH or mouse CR2, on the other hand, had little effects in both assay systems. This experiment demonstrates a clear advantage of using CR2-FH to inhibit alternative complement pathway, particularly when the classical complement pathway is not involved.

To further demonstrate that the inhibition of C3b deposition observed with CR2-FH was due to inhibition of the alternative pathway, we studied the effects of CR2-FH on C3b deposition in the absence of the classical pathway (C4⁻/C4⁻ mice). Calcium inhibits the lectin complement pathway. FIG. 24 shows a titration curve of mouse CR2-FH in calcium sufficient buffer using serum from C4⁻/C4⁻ knockout mice. As shown in the figure, CR2-FH significantly inhibits C3b deposition at the concentration of 0.5 μg/μl.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it is apparent to those skilled in the art that certain minor changes and modifications will be practiced. Therefore, the description and examples should not be construed as limiting the scope of the invention.

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| Met | Ser | Asp | Ser | Tyr | Gln | Tyr | Gly | Glu | Glu | Val | Thr | Tyr | Lys | Cys | Phe |
| 945 | | | | | 950 | | | | | 955 | | | | | 960 |
| Glu | Gly | Phe | Gly | Ile | Asp | Gly | Pro | Ala | Ile | Ala | Lys | Cys | Leu | Gly | Glu |
| | | | | 965 | | | | | 970 | | | | | 975 | |
| Lys | Trp | Ser | His | Pro | Pro | Ser | Cys | Ile | Lys | Thr | Asp | Cys | Leu | Ser | Leu |
| | | | 980 | | | | | 985 | | | | | 990 | | |
| Pro | Ser | Phe | Glu | Asn | Ala | Ile | Pro | Met | Gly | Glu | Lys | Lys | Asp | Val | Tyr |
| | | 995 | | | | | 1000 | | | | | | 1005 | | |
| Lys | Ala | Gly | Glu | Gln | Val | Thr | Tyr | Thr | Cys | Ala | Thr | Tyr | Tyr | Lys | |
| | 1010 | | | | | | 1015 | | | | | 1020 | | | |
| Met | Asp | Gly | Ala | Ser | Asn | Val | Thr | Cys | Ile | Asn | Ser | Arg | Trp | Thr | |
| | 1025 | | | | | 1030 | | | | | 1035 | | | | |
| Gly | Arg | Pro | Thr | Cys | Arg | Asp | Thr | Ser | Cys | Val | Asn | Pro | Pro | Thr | |
| | 1040 | | | | | 1045 | | | | | 1050 | | | | |
| Val | Gln | Asn | Ala | Tyr | Ile | Val | Ser | Arg | Gln | Met | Ser | Lys | Tyr | Pro | |

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| | | |
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| 1055 | 1060 | 1065 |
| Ser Gly Glu Arg Val Arg Tyr Gln Cys Arg Ser Pro Tyr Glu Met 1070 1075 1080 | | |
| Phe Gly Asp Glu Glu Val Met Cys Leu Asn Gly Asn Trp Thr Glu 1085 1090 1095 | | |
| Pro Pro Gln Cys Lys Asp Ser Thr Gly Lys Cys Gly Pro Pro Pro 1100 1105 1110 | | |
| Pro Ile Asp Asn Gly Asp Ile Thr Ser Phe Pro Leu Ser Val Tyr 1115 1120 1125 | | |
| Ala Pro Ala Ser Ser Val Glu Tyr Gln Cys Gln Asn Leu Tyr Gln 1130 1135 1140 | | |
| Leu Glu Gly Asn Lys Arg Ile Thr Cys Arg Asn Gly Gln Trp Ser 1145 1150 1155 | | |
| Glu Pro Pro Lys Cys Leu His Pro Cys Val Ile Ser Arg Glu Ile 1160 1165 1170 | | |
| Met Glu Asn Tyr Asn Ile Ala Leu Arg Trp Thr Ala Lys Gln Lys 1175 1180 1185 | | |
| Leu Tyr Ser Arg Thr Gly Glu Ser Val Glu Phe Val Cys Lys Arg 1190 1195 1200 | | |
| Gly Tyr Arg Leu Ser Ser Arg Ser His Thr Leu Arg Thr Thr Cys 1205 1210 1215 | | |
| Trp Asp Gly Lys Leu Glu Tyr Pro Thr Cys Ala Lys Arg 1220 1225 1230 | | |

<210> SEQ ID NO 3
 <211> LENGTH: 570
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 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <221> NAME/KEY: VARIANT
 <222> LOCATION: (197)..(197)
 <223> OTHER INFORMATION: Xaa = Unknown or Other

<400> SEQUENCE: 3

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| Tyr Ser Thr Pro Ile Ala Val Gly Thr Val Ile Arg Tyr Ser Cys Ser 20 25 30 |
| Gly Thr Phe Arg Leu Ile Gly Glu Lys Ser Leu Leu Cys Ile Thr Lys 35 40 45 |
| Asp Lys Val Asp Gly Thr Trp Asp Lys Pro Ala Pro Lys Cys Glu Tyr 50 55 60 |
| Phe Asn Lys Tyr Ser Ser Cys Pro Glu Pro Ile Val Pro Gly Gly Tyr 65 70 75 80 |
| Lys Ile Arg Gly Ser Thr Pro Tyr Arg His Gly Asp Ser Val Thr Phe 85 90 95 |
| Ala Cys Lys Thr Asn Phe Ser Met Asn Gly Asn Lys Ser Val Trp Cys 100 105 110 |
| Gln Ala Asn Asn Ile Asn Asn Met Trp Gly Pro Thr Arg Leu Pro Thr 115 120 125 |
| Cys Val Ser Val Phe Pro Leu Glu Cys Pro Ala Leu Pro Met Ile His 130 135 140 |
| Asn Gly His His Thr Ser Glu Asn Val Gly Ser Ile Ala Pro Gly Leu 145 150 155 160 |
| Ser Val Thr Tyr Ser Cys Glu Ser Gly Tyr Leu Leu Val Gly Glu Lys 165 170 175 |

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Ile Ile Asn Cys Leu Ser Ser Gly Lys Trp Ser Ala Val Pro Pro Thr
 180 185 190
 Cys Glu Glu Ala Xaa Cys Lys Ser Leu Gly Arg Phe Pro Asn Gly Lys
 195 200 205
 Val Lys Glu Pro Pro Ile Leu Arg Val Gly Val Thr Ala Asn Phe Phe
 210 215 220
 Cys Asp Glu Gly Tyr Arg Leu Gln Gly Pro Pro Ser Ser Arg Cys Val
 225 230 235 240
 Ile Ala Gly Gln Gly Val Ala Trp Thr Lys Met Pro Val Cys Gly Gly
 245 250 255
 Gly Gly Ser Gly Gly Gly Gly Ser Cys Val Ala Glu Asp Cys Asn Glu
 260 265 270
 Leu Pro Pro Arg Arg Asn Thr Glu Ile Leu Thr Gly Ser Trp Ser Asp
 275 280 285
 Gln Thr Tyr Pro Glu Gly Thr Gln Ala Ile Tyr Lys Cys Arg Pro Gly
 290 295 300
 Tyr Arg Ser Leu Gly Asn Val Ile Met Val Cys Arg Lys Gly Glu Trp
 305 310 315 320
 Val Ala Leu Asn Pro Leu Arg Lys Cys Gln Lys Arg Pro Cys Gly His
 325 330 335
 Pro Gly Asp Thr Pro Phe Gly Thr Phe Thr Leu Thr Gly Gly Asn Val
 340 345 350
 Phe Glu Tyr Gly Val Lys Ala Val Tyr Thr Cys Asn Glu Gly Tyr Gln
 355 360 365
 Leu Leu Gly Glu Ile Asn Tyr Arg Glu Cys Asp Thr Asp Gly Trp Thr
 370 375 380
 Asn Asp Ile Pro Ile Cys Glu Val Val Lys Cys Leu Pro Val Thr Ala
 385 390 395 400
 Pro Glu Asn Gly Lys Ile Val Ser Ser Ala Met Glu Pro Asp Arg Glu
 405 410 415
 Tyr His Phe Gly Gln Ala Val Arg Phe Val Cys Asn Ser Gly Tyr Lys
 420 425 430
 Ile Glu Gly Asp Glu Glu Met His Cys Ser Asp Asp Gly Phe Trp Ser
 435 440 445
 Lys Glu Lys Pro Lys Cys Val Glu Ile Ser Cys Lys Ser Pro Asp Val
 450 455 460
 Ile Asn Gly Ser Pro Ile Ser Gln Lys Ile Ile Tyr Lys Glu Asn Glu
 465 470 475 480
 Arg Phe Gln Tyr Lys Cys Asn Met Gly Tyr Glu Tyr Ser Glu Arg Gly
 485 490 495
 Asp Ala Val Cys Thr Glu Ser Gly Trp Arg Pro Leu Pro Ser Cys Glu
 500 505 510
 Glu Lys Ser Cys Asp Asn Pro Tyr Ile Pro Asn Gly Asp Tyr Ser Pro
 515 520 525
 Leu Arg Ile Lys His Arg Thr Gly Asp Glu Ile Thr Tyr Gln Cys Arg
 530 535 540
 Asn Gly Phe Tyr Pro Ala Thr Arg Gly Asn Thr Ala Lys Cys Thr Ser
 545 550 555 560
 Thr Gly Trp Ile Pro Ala Pro Arg Cys Thr
 565 570

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<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 4

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aaaagtctat tatgcataac taaagacaaa gtggatggaa cctgggataa acctgctcct   180
aaatgtgaat atttcaataa atattcttct tgccctgagc ccatagtacc aggaggatac   240
aaaattagag gctctacacc ctacagacat ggtgattctg tgacatttgc ctgtaaaacc   300
aacttctcca tgaacggaaa caagtctggt tggtgtcaag caaataatat aaataatatg   360
tgggggccga cacgactacc aacctgtgta agtgtttcc ctctcgagtg tccagcactt   420
cctatgatcc acaatggaca tcacacaagt gagaatggtg gctccattgc tccaggattg   480
tctgtgactt acagctgtga atctggttac ttgcttggtg gagaaaagat cattaactgt   540
ttgtcttcgg gaaaatggag tgctgtcccc cccacatgtg aagaggcacs ctgtaaatct   600
ctaggacgat ttcccaatgg gaaggtaaag gagcctccaa ttctccgggt tgggtgtaact   660
gcaaactttt tctgtgatga agggatcga ctgcaaggcc caccttctag tcggtgtgta   720
attgctggac agggagttgc ttggacaaa atgccagtat gtggcggagg tgggtcgggt   780
ggcggcggat cttgtgtagc agaagattgc aatgaacttc ctccaagaag aaatacagaa   840
attctgacag gttcctggtc tgaccaaaca tatccagaag gcaccaggc tatctataaa   900
tgccgcctg gatatagatc tcttgaaaat gtaataatgg tatgcaggaa gggagaatgg   960
gttgctctta atccattaag gaaatgtcag aaaaggccct gtggacatcc tggagatact  1020
ccttttggtg cttttaccct tacaggagga aatgtgtttg aatattggtg aaaagctgtg  1080
tatacatgta atgaggggta tcaattgcta ggtgagatta attacctga atgtgacaca  1140
gatggatgga ccaatgatat tcctatatgt gaagttgtga agtgtttacc agtgacagca  1200
ccagagaatg gaaaaattgt cagtagtgca atggaaccag atcgggaata ccattttgga  1260
caagcagtac ggtttgtatg taactcaggc tacaagattg aaggagatga agaaatgcat  1320
tggtcagacg atggtttttg gagtaaagag aaaccaagt gtgtggaaat ttcattgcaa  1380
tccccagatg ttataaatgg atctcctata tctcagaaga ttatttataa ggagaatgaa  1440
cgatttcaat ataaatgtaa catgggttat gaatacagtg aaagaggaga tgctgtatgc  1500
actgaatctg gatggcgtcc gttgccttca tgtgaagaaa aatcatgtga taatccttat  1560
attccaaatg gtgactactc acctttaaagg attaaacaca gaactggaga tgaaatcacg  1620
taccagtgta gaaatggttt ttatcctgca acccggggaa atacagcaa atgcacaagt  1680
actggctgga tacctgctcc gagatgtacc t                                     1711

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<210> SEQ ID NO 5
<211> LENGTH: 560
<212> TYPE: PRT
<213> ORGANISM: Murine
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<222> LOCATION: (252)..(253)
<223> OTHER INFORMATION: Xaa = Unknown or Other
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (252)..(254)
<223> OTHER INFORMATION: Optional Linker

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<400> SEQUENCE: 5

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 20 25 30
 Gly Thr Phe Arg Leu Ile Gly Glu Lys Ser Leu Leu Cys Ile Thr Lys
 35 40 45
 Asp Lys Val Asp Gly Thr Trp Asp Lys Pro Ala Pro Lys Cys Glu Tyr
 50 55 60
 Phe Asn Lys Tyr Ser Ser Cys Pro Glu Pro Ile Val Pro Gly Gly Tyr
 65 70 75 80
 Lys Ile Arg Gly Ser Thr Pro Tyr Arg His Gly Asp Ser Val Thr Phe
 85 90 95
 Ala Cys Lys Thr Asn Phe Ser Met Asn Gly Asn Lys Ser Val Trp Cys
 100 105 110
 Gln Ala Asn Asn Met Trp Gly Pro Thr Arg Leu Pro Thr Cys Val Ser
 115 120 125
 Val Phe Pro Leu Glu Cys Pro Ala Leu Pro Met Ile His Asn Gly His
 130 135 140
 His Thr Ser Glu Asn Val Gly Ser Ile Ala Pro Gly Leu Ser Val Thr
 145 150 155 160
 Tyr Ser Cys Glu Ser Gly Tyr Leu Leu Val Gly Glu Lys Ile Ile Asn
 165 170 175
 Cys Leu Ser Ser Gly Lys Trp Ser Ala Val Pro Pro Thr Cys Glu Glu
 180 185 190
 Ala Arg Cys Lys Ser Leu Gly Arg Phe Pro Asn Gly Lys Val Lys Glu
 195 200 205
 Pro Pro Ile Leu Arg Val Gly Val Thr Ala Asn Phe Phe Cys Asp Glu
 210 215 220
 Gly Tyr Arg Leu Gln Gly Pro Pro Ser Ser Arg Cys Val Ile Ala Gly
 225 230 235 240
 Gln Gly Val Ala Trp Thr Lys Met Pro Val Cys Xaa Xaa Xaa Cys Val
 245 250 255
 Ala Glu Asp Cys Asn Glu Leu Pro Pro Arg Arg Asn Thr Glu Ile Leu
 260 265 270
 Thr Gly Ser Trp Ser Asp Gln Thr Tyr Pro Glu Gly Thr Gln Ala Ile
 275 280 285
 Tyr Lys Cys Arg Pro Gly Tyr Arg Ser Leu Gly Asn Val Ile Met Val
 290 295 300
 Cys Arg Lys Gly Glu Trp Val Ala Leu Asn Pro Leu Arg Lys Cys Gln
 305 310 315 320
 Lys Arg Pro Cys Gly His Pro Gly Asp Thr Pro Phe Gly Thr Phe Thr
 325 330 335
 Leu Thr Gly Gly Asn Val Phe Glu Tyr Gly Val Lys Ala Val Tyr Thr
 340 345 350
 Cys Asn Glu Gly Tyr Gln Leu Leu Gly Glu Ile Asn Tyr Arg Glu Cys
 355 360 365
 Asp Thr Asp Gly Trp Thr Asn Asp Ile Pro Ile Cys Glu Val Val Lys
 370 375 380
 Cys Leu Pro Val Thr Ala Pro Glu Asn Gly Lys Ile Val Ser Ser Ala
 385 390 395 400
 Met Glu Pro Asp Arg Glu Tyr His Phe Gly Gln Ala Val Arg Phe Val
 405 410 415
 Cys Asn Ser Gly Tyr Lys Ile Glu Gly Asp Glu Glu Met His Cys Ser

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| 420 | | | | | 425 | | | | | 430 | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asp | Asp | Gly | Phe | Trp | Ser | Lys | Glu | Lys | Pro | Lys | Cys | Val | Glu | Ile | Ser |
| | | 435 | | | | | 440 | | | | | 445 | | | |
| Cys | Lys | Ser | Pro | Asp | Val | Ile | Asn | Gly | Ser | Pro | Ile | Ser | Gln | Lys | Ile |
| | | 450 | | | | | 455 | | | | | 460 | | | |
| Ile | Tyr | Lys | Glu | Asn | Glu | Arg | Phe | Gln | Tyr | Lys | Cys | Asn | Met | Gly | Tyr |
| | | | | | | | 470 | | | | | 475 | | | 480 |
| Glu | Tyr | Ser | Glu | Arg | Gly | Asp | Ala | Val | Cys | Thr | Glu | Ser | Gly | Trp | Arg |
| | | | | | | | 485 | | | | | 490 | | | 495 |
| Pro | Leu | Pro | Ser | Cys | Glu | Glu | Lys | Ser | Cys | Asp | Asn | Pro | Tyr | Ile | Pro |
| | | | | | | | 500 | | | | | 505 | | | 510 |
| Asn | Gly | Asp | Tyr | Ser | Pro | Leu | Arg | Ile | Lys | His | Arg | Thr | Gly | Asp | Glu |
| | | | | | | | 515 | | | | | 520 | | | 525 |
| Ile | Thr | Tyr | Gln | Cys | Arg | Asn | Gly | Phe | Tyr | Pro | Ala | Thr | Arg | Gly | Asn |
| | | | | | | | 530 | | | | | 535 | | | 540 |
| Thr | Ala | Lys | Cys | Thr | Ser | Thr | Gly | Trp | Ile | Pro | Ala | Pro | Arg | Cys | Thr |
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<210> SEQ ID NO 6
 <211> LENGTH: 560
 <212> TYPE: PRT
 <213> ORGANISM: Murine
 <220> FEATURE:
 <221> NAME/KEY: VARIANT
 <222> LOCATION: (252)..(253)
 <223> OTHER INFORMATION: Xaa = Unknown or Other
 <220> FEATURE:
 <221> NAME/KEY: VARIANT
 <222> LOCATION: (252)..(254)
 <223> OTHER INFORMATION: Optional Linker

<400> SEQUENCE: 6

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| 1 | | | | 5 | | | | | 10 | | | | | 15 | |
| Tyr | Ser | Thr | Pro | Ile | Ala | Val | Gly | Thr | Val | Ile | Arg | Tyr | Ser | Cys | Ser |
| | | | | 20 | | | | 25 | | | | | | 30 | |
| Gly | Thr | Phe | Arg | Leu | Ile | Gly | Glu | Lys | Ser | Leu | Leu | Cys | Ile | Thr | Lys |
| | | | | 35 | | | | 40 | | | | | | 45 | |
| Asp | Lys | Val | Asp | Gly | Thr | Trp | Asp | Lys | Pro | Ala | Pro | Lys | Cys | Glu | Tyr |
| | | | | 50 | | | | 55 | | | | | | 60 | |
| Phe | Asn | Lys | Tyr | Ser | Ser | Cys | Pro | Glu | Pro | Ile | Val | Pro | Gly | Gly | Tyr |
| | | | | | | | | 70 | | | | | | 75 | 80 |
| Lys | Ile | Arg | Gly | Ser | Thr | Pro | Tyr | Arg | His | Gly | Asp | Ser | Val | Thr | Phe |
| | | | | | | | | 85 | | | | | | 90 | 95 |
| Ala | Cys | Lys | Thr | Asn | Phe | Ser | Met | Asn | Gly | Asn | Lys | Ser | Val | Trp | Cys |
| | | | | | | | | 100 | | | | | | 105 | 110 |
| Gln | Ala | Asn | Asn | Met | Trp | Gly | Pro | Thr | Arg | Leu | Pro | Thr | Cys | Val | Ser |
| | | | | | | | | 115 | | | | | | 120 | 125 |
| Val | Phe | Pro | Leu | Glu | Cys | Pro | Ala | Leu | Pro | Met | Ile | His | Asn | Gly | His |
| | | | | | | | | 130 | | | | | | 135 | 140 |
| His | Thr | Ser | Glu | Asn | Val | Gly | Ser | Ile | Ala | Pro | Gly | Leu | Ser | Val | Thr |
| | | | | | | | | 145 | | | | | | 150 | 155 |
| Tyr | Ser | Cys | Glu | Ser | Gly | Tyr | Leu | Leu | Val | Gly | Glu | Lys | Ile | Ile | Asn |
| | | | | | | | | 165 | | | | | | 170 | 175 |
| Cys | Leu | Ser | Ser | Gly | Lys | Trp | Ser | Ala | Val | Pro | Pro | Thr | Cys | Glu | Glu |
| | | | | | | | | 180 | | | | | | 185 | 190 |
| Ala | Arg | Cys | Lys | Ser | Leu | Gly | Arg | Phe | Pro | Asn | Gly | Lys | Val | Lys | Glu |

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| 195 | | | | | 200 | | | | | 205 | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Pro | Ile | Leu | Arg | Val | Gly | Val | Thr | Ala | Asn | Phe | Phe | Cys | Asp | Glu |
| | 210 | | | | | 215 | | | | | 220 | | | | |
| Gly | Tyr | Arg | Leu | Gln | Gly | Pro | Pro | Ser | Ser | Arg | Cys | Val | Ile | Ala | Gly |
| | 225 | | | | | 230 | | | | | 235 | | | | 240 |
| Gln | Gly | Val | Ala | Trp | Thr | Lys | Met | Pro | Val | Cys | Xaa | Xaa | Xaa | Cys | Val |
| | | | | 245 | | | | | 250 | | | | | 255 | |
| Ala | Glu | Asp | Cys | Asn | Glu | Leu | Pro | Pro | Arg | Arg | Asn | Thr | Glu | Ile | Leu |
| | | | 260 | | | | | 265 | | | | | 270 | | |
| Thr | Gly | Ser | Trp | Ser | Asp | Gln | Thr | Tyr | Pro | Glu | Gly | Thr | Gln | Ala | Ile |
| | | 275 | | | | | 280 | | | | | 285 | | | |
| Tyr | Lys | Cys | Arg | Pro | Gly | Tyr | Arg | Ser | Leu | Gly | Asn | Ile | Ile | Met | Val |
| | 290 | | | | | 295 | | | | | 300 | | | | |
| Cys | Arg | Lys | Gly | Glu | Trp | Val | Ala | Leu | Asn | Pro | Leu | Arg | Lys | Cys | Gln |
| | 305 | | | | | 310 | | | | | 315 | | | | 320 |
| Lys | Arg | Pro | Cys | Gly | His | Pro | Gly | Asp | Thr | Pro | Phe | Gly | Thr | Phe | Thr |
| | | | | 325 | | | | | 330 | | | | | 335 | |
| Leu | Thr | Gly | Gly | Asn | Val | Phe | Glu | Tyr | Gly | Val | Lys | Ala | Val | Tyr | Thr |
| | | | 340 | | | | | 345 | | | | | 350 | | |
| Cys | Asn | Glu | Gly | Tyr | Gln | Leu | Leu | Gly | Glu | Ile | Asn | Tyr | Arg | Glu | Cys |
| | | 355 | | | | | 360 | | | | | 365 | | | |
| Asp | Thr | Asp | Gly | Trp | Thr | Asn | Asp | Ile | Pro | Ile | Cys | Glu | Val | Val | Lys |
| | 370 | | | | | 375 | | | | | 380 | | | | |
| Cys | Leu | Pro | Val | Thr | Ala | Pro | Glu | Asn | Gly | Lys | Ile | Val | Ser | Ser | Ala |
| | 385 | | | | | 390 | | | | | 395 | | | | 400 |
| Met | Glu | Pro | Asp | Arg | Glu | Tyr | His | Phe | Gly | Gln | Ala | Val | Arg | Phe | Val |
| | | | | 405 | | | | | 410 | | | | | 415 | |
| Cys | Asn | Ser | Gly | Tyr | Lys | Ile | Glu | Gly | Asp | Glu | Glu | Met | His | Cys | Ser |
| | | | 420 | | | | | 425 | | | | | 430 | | |
| Asp | Asp | Gly | Phe | Trp | Ser | Lys | Glu | Lys | Pro | Lys | Cys | Val | Glu | Ile | Ser |
| | 435 | | | | | 440 | | | | | | 445 | | | |
| Cys | Lys | Ser | Pro | Asp | Val | Ile | Asn | Gly | Ser | Pro | Ile | Ser | Gln | Lys | Ile |
| | 450 | | | | | 455 | | | | | 460 | | | | |
| Ile | Tyr | Lys | Glu | Asn | Glu | Arg | Phe | Gln | Tyr | Lys | Cys | Asn | Met | Gly | Tyr |
| | 465 | | | | | 470 | | | | | 475 | | | | 480 |
| Glu | Tyr | Ser | Glu | Arg | Gly | Asp | Ala | Val | Cys | Thr | Glu | Ser | Gly | Trp | Arg |
| | | | | 485 | | | | | 490 | | | | | 495 | |
| Pro | Leu | Pro | Ser | Cys | Glu | Glu | Lys | Ser | Cys | Asp | Asn | Pro | Tyr | Ile | Pro |
| | | | 500 | | | | | 505 | | | | | 510 | | |
| Asn | Gly | Asp | Tyr | Ser | Pro | Leu | Arg | Ile | Lys | His | Arg | Thr | Gly | Asp | Glu |
| | | 515 | | | | | 520 | | | | | 525 | | | |
| Ile | Thr | Tyr | Gln | Cys | Arg | Asn | Gly | Phe | Tyr | Pro | Ala | Thr | Arg | Gly | Asn |
| | 530 | | | | | 535 | | | | | 540 | | | | |
| Thr | Ala | Lys | Cys | Thr | Ser | Thr | Gly | Trp | Ile | Pro | Ala | Pro | Arg | Cys | Thr |
| | 545 | | | | | 550 | | | | | 555 | | | | 560 |

<210> SEQ ID NO 7
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 <213> ORGANISM: Murine
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 <222> LOCATION: (197)..(255)
 <223> OTHER INFORMATION: Xaa = Unknown or Other
 <220> FEATURE:
 <221> NAME/KEY: VARIANT

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<222> LOCATION: (255)..(257)

<223> OTHER INFORMATION: Optional Linker

<400> SEQUENCE: 7

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20          25          30
Gly Thr Phe Arg Leu Ile Gly Glu Lys Ser Leu Leu Cys Ile Thr Lys
35          40          45
Asp Lys Val Asp Gly Thr Trp Asp Lys Pro Ala Pro Lys Cys Glu Tyr
50          55          60
Phe Asn Lys Tyr Ser Ser Cys Pro Glu Pro Ile Val Pro Gly Gly Tyr
65          70          75          80
Lys Ile Arg Gly Ser Thr Pro Tyr Arg His Gly Asp Ser Val Thr Phe
85          90          95
Ala Cys Lys Thr Asn Phe Ser Met Asn Gly Asn Lys Ser Val Trp Cys
100         105         110
Gln Ala Asn Asn Ile Asn Asn Met Trp Gly Pro Thr Arg Leu Pro Thr
115         120         125
Cys Val Ser Val Phe Pro Leu Glu Cys Pro Ala Leu Pro Met Ile His
130         135         140
Asn Gly His His Thr Ser Glu Asn Val Gly Ser Ile Ala Pro Gly Leu
145         150         155         160
Ser Val Thr Tyr Ser Cys Glu Ser Gly Tyr Leu Leu Val Gly Glu Lys
165         170         175
Ile Ile Asn Cys Leu Ser Ser Gly Lys Trp Ser Ala Val Pro Pro Thr
180         185         190
Cys Glu Glu Ala Xaa Cys Lys Ser Leu Gly Arg Phe Pro Asn Gly Lys
195         200         205
Val Lys Glu Pro Pro Ile Leu Arg Val Gly Val Thr Ala Asn Phe Phe
210         215         220
Cys Asp Glu Gly Tyr Arg Leu Gln Gly Pro Pro Ser Ser Arg Cys Val
225         230         235         240
Ile Ala Gly Gln Gly Val Ala Trp Thr Lys Met Pro Val Cys Xaa Xaa
245         250         255
Xaa Glu Asp Cys Asn Glu Leu Pro Pro Arg Arg Asn Thr Glu Ile Leu
260         265         270
Thr Gly Ser Trp Ser Asp Gln Thr Tyr Pro Glu Gly Thr Gln Ala Ile
275         280         285
Tyr Lys Cys Arg Pro Gly Tyr Arg Ser Leu Gly Asn Val Ile Met Val
290         295         300
Cys Arg Lys Gly Glu Trp Val Ala Leu Asn Pro Leu Arg Lys Cys Gln
305         310         315         320
Lys Arg Pro Cys Gly His Pro Gly Asp Thr Pro Phe Gly Thr Phe Thr
325         330         335
Leu Thr Gly Gly Asn Val Phe Glu Tyr Gly Val Lys Ala Val Tyr Thr
340         345         350
Cys Asn Glu Gly Tyr Gln Leu Leu Gly Glu Ile Asn Tyr Arg Glu Cys
355         360         365
Asp Thr Asp Gly Trp Thr Asn Asp Ile Pro Ile Cys Glu Val Val Lys
370         375         380
Cys Leu Pro Val Thr Ala Pro Glu Asn Gly Lys Ile Val Ser Ser Ala
385         390         395         400

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Met Glu Pro Asp Arg Glu Tyr His Phe Gly Gln Ala Val Arg Phe Val
 405 410 415

Cys Asn Ser Gly Tyr Lys Ile Glu Gly Asp Glu Glu Met His Cys Ser
 420 425 430

Asp Asp Gly Phe Trp Ser Lys Glu Lys Pro Lys Cys Val Glu Ile Ser
 435 440 445

Cys Lys Ser Pro Asp Val Ile Asn Gly Ser Pro Ile Ser Gln Lys Ile
 450 455 460

Ile Tyr Lys Glu Asn Glu Arg Phe Gln Tyr Lys Cys Asn Met Gly Tyr
 465 470 475 480

Glu Tyr Ser Glu Arg Gly Asp Ala Val Cys Thr Glu Ser Gly Trp Arg
 485 490 495

Pro Leu Pro Ser Cys Glu Glu Lys Ser Cys Asp Asn Pro Tyr Ile Pro
 500 505 510

Asn Gly Asp Tyr Ser Pro Leu Arg Ile Lys His Arg Thr Gly Asp Glu
 515 520 525

Ile Thr Tyr Gln Cys Arg Asn Gly Phe Tyr Pro Ala Thr Arg Gly Asn
 530 535 540

Thr Ala Lys Cys Thr Ser Thr Gly Trp Ile Pro Ala Pro Arg Cys Thr
 545 550 555 560

<210> SEQ ID NO 8
 <211> LENGTH: 560
 <212> TYPE: PRT
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 <221> NAME/KEY: VARIANT
 <222> LOCATION: (197)..(255)
 <223> OTHER INFORMATION: Xaa = Unknown or Other
 <220> FEATURE:
 <221> NAME/KEY: VARIANT
 <222> LOCATION: (255)..(257)
 <223> OTHER INFORMATION: Optional Linker

<400> SEQUENCE: 8

Ile Ser Cys Gly Ser Pro Pro Pro Ile Leu Asn Gly Arg Ile Ser Tyr
 1 5 10 15

Tyr Ser Thr Pro Ile Ala Val Gly Thr Val Ile Arg Tyr Ser Cys Ser
 20 25 30

Gly Thr Phe Arg Leu Ile Gly Glu Lys Ser Leu Leu Cys Ile Thr Lys
 35 40 45

Asp Lys Val Asp Gly Thr Trp Asp Lys Pro Ala Pro Lys Cys Glu Tyr
 50 55 60

Phe Asn Lys Tyr Ser Ser Cys Pro Glu Pro Ile Val Pro Gly Gly Tyr
 65 70 75 80

Lys Ile Arg Gly Ser Thr Pro Tyr Arg His Gly Asp Ser Val Thr Phe
 85 90 95

Ala Cys Lys Thr Asn Phe Ser Met Asn Gly Asn Lys Ser Val Trp Cys
 100 105 110

Gln Ala Asn Asn Ile Asn Asn Met Trp Gly Pro Thr Arg Leu Pro Thr
 115 120 125

Cys Val Ser Val Phe Pro Leu Glu Cys Pro Ala Leu Pro Met Ile His
 130 135 140

Asn Gly His His Thr Ser Glu Asn Val Gly Ser Ile Ala Pro Gly Leu
 145 150 155 160

Ser Val Thr Tyr Ser Cys Glu Ser Gly Tyr Leu Leu Val Gly Glu Lys
 165 170 175

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Ile Ile Asn Cys Leu Ser Ser Gly Lys Trp Ser Ala Val Pro Pro Thr
      180                               185                190
Cys Glu Glu Ala Xaa Cys Lys Ser Leu Gly Arg Phe Pro Asn Gly Lys
      195                               200                205
Val Lys Glu Pro Pro Ile Leu Arg Val Gly Val Thr Ala Asn Phe Phe
      210                               215                220
Cys Asp Glu Gly Tyr Arg Leu Gln Gly Pro Pro Ser Ser Arg Cys Val
      225                               230                235                240
Ile Ala Gly Gln Gly Val Ala Trp Thr Lys Met Pro Val Cys Xaa Xaa
      245                               250                255
Xaa Glu Asp Cys Asn Glu Leu Pro Pro Arg Arg Asn Thr Glu Ile Leu
      260                               265                270
Thr Gly Ser Trp Ser Asp Gln Thr Tyr Pro Glu Gly Thr Gln Ala Ile
      275                               280                285
Tyr Lys Cys Arg Pro Gly Tyr Arg Ser Leu Gly Asn Ile Ile Met Val
      290                               295                300
Cys Arg Lys Gly Glu Trp Val Ala Leu Asn Pro Leu Arg Lys Cys Gln
      305                               310                315                320
Lys Arg Pro Cys Gly His Pro Gly Asp Thr Pro Phe Gly Thr Phe Thr
      325                               330                335
Leu Thr Gly Gly Asn Val Phe Glu Tyr Gly Val Lys Ala Val Tyr Thr
      340                               345                350
Cys Asn Glu Gly Tyr Gln Leu Leu Gly Glu Ile Asn Tyr Arg Glu Cys
      355                               360                365
Asp Thr Asp Gly Trp Thr Asn Asp Ile Pro Ile Cys Glu Val Val Lys
      370                               375                380
Cys Leu Pro Val Thr Ala Pro Glu Asn Gly Lys Ile Val Ser Ser Ala
      385                               390                395                400
Met Glu Pro Asp Arg Glu Tyr His Phe Gly Gln Ala Val Arg Phe Val
      405                               410                415
Cys Asn Ser Gly Tyr Lys Ile Glu Gly Asp Glu Glu Met His Cys Ser
      420                               425                430
Asp Asp Gly Phe Trp Ser Lys Glu Lys Pro Lys Cys Val Glu Ile Ser
      435                               440                445
Cys Lys Ser Pro Asp Val Ile Asn Gly Ser Pro Ile Ser Gln Lys Ile
      450                               455                460
Ile Tyr Lys Glu Asn Glu Arg Phe Gln Tyr Lys Cys Asn Met Gly Tyr
      465                               470                475                480
Glu Tyr Ser Glu Arg Gly Asp Ala Val Cys Thr Glu Ser Gly Trp Arg
      485                               490                495
Pro Leu Pro Ser Cys Glu Glu Lys Ser Cys Asp Asn Pro Tyr Ile Pro
      500                               505                510
Asn Gly Asp Tyr Ser Pro Leu Arg Ile Lys His Arg Thr Gly Asp Glu
      515                               520                525
Ile Thr Tyr Gln Cys Arg Asn Gly Phe Tyr Pro Ala Thr Arg Gly Asn
      530                               535                540
Thr Ala Lys Cys Thr Ser Thr Gly Trp Ile Pro Ala Pro Arg Cys Thr
      545                               550                555                560

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<210> SEQ ID NO 9
<211> LENGTH: 557
<212> TYPE: PRT
<213> ORGANISM: Murine
<220> FEATURE:

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<221> NAME/KEY: VARIANT
<222> LOCATION: (252)..(253)
<223> OTHER INFORMATION: Xaa = Unknown or Other
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (252)..(254)
<223> OTHER INFORMATION: Optional Linker

<400> SEQUENCE: 9

Ile Ser Cys Gly Ser Pro Pro Pro Ile Leu Asn Gly Arg Ile Ser Tyr
1          5          10          15
Tyr Ser Thr Pro Ile Ala Val Gly Thr Val Ile Arg Tyr Ser Cys Ser
20          25          30
Gly Thr Phe Arg Leu Ile Gly Glu Lys Ser Leu Leu Cys Ile Thr Lys
35          40          45
Asp Lys Val Asp Gly Thr Trp Asp Lys Pro Ala Pro Lys Cys Glu Tyr
50          55          60
Phe Asn Lys Tyr Ser Ser Cys Pro Glu Pro Ile Val Pro Gly Gly Tyr
65          70          75          80
Lys Ile Arg Gly Ser Thr Pro Tyr Arg His Gly Asp Ser Val Thr Phe
85          90          95
Ala Cys Lys Thr Asn Phe Ser Met Asn Gly Asn Lys Ser Val Trp Cys
100         105         110
Gln Ala Asn Asn Met Trp Gly Pro Thr Arg Leu Pro Thr Cys Val Ser
115         120         125
Val Phe Pro Leu Glu Cys Pro Ala Leu Pro Met Ile His Asn Gly His
130         135         140
His Thr Ser Glu Asn Val Gly Ser Ile Ala Pro Gly Leu Ser Val Thr
145         150         155         160
Tyr Ser Cys Glu Ser Gly Tyr Leu Leu Val Gly Glu Lys Ile Ile Asn
165         170         175
Cys Leu Ser Ser Gly Lys Trp Ser Ala Val Pro Pro Thr Cys Glu Glu
180         185         190
Ala Arg Cys Lys Ser Leu Gly Arg Phe Pro Asn Gly Lys Val Lys Glu
195         200         205
Pro Pro Ile Leu Arg Val Gly Val Thr Ala Asn Phe Phe Cys Asp Glu
210         215         220
Gly Tyr Arg Leu Gln Gly Pro Pro Ser Ser Arg Cys Val Ile Ala Gly
225         230         235         240
Gln Gly Val Ala Trp Thr Lys Met Pro Val Cys Xaa Xaa Xaa Glu Asp
245         250         255
Cys Asn Glu Leu Pro Pro Arg Arg Asn Thr Glu Ile Leu Thr Gly Ser
260         265         270
Trp Ser Asp Gln Thr Tyr Pro Glu Gly Thr Gln Ala Ile Tyr Lys Cys
275         280         285
Arg Pro Gly Tyr Arg Ser Leu Gly Asn Val Ile Met Val Cys Arg Lys
290         295         300
Gly Glu Trp Val Ala Leu Asn Pro Leu Arg Lys Cys Gln Lys Arg Pro
305         310         315         320
Cys Gly His Pro Gly Asp Thr Pro Phe Gly Thr Phe Thr Leu Thr Gly
325         330         335
Gly Asn Val Phe Glu Tyr Gly Val Lys Ala Val Tyr Thr Cys Asn Glu
340         345         350
Gly Tyr Gln Leu Leu Gly Glu Ile Asn Tyr Arg Glu Cys Asp Thr Asp
355         360         365

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Gly Trp Thr Asn Asp Ile Pro Ile Cys Glu Val Val Lys Cys Leu Pro
 370 375 380
 Val Thr Ala Pro Glu Asn Gly Lys Ile Val Ser Ser Ala Met Glu Pro
 385 390 395 400
 Asp Arg Glu Tyr His Phe Gly Gln Ala Val Arg Phe Val Cys Asn Ser
 405 410 415
 Gly Tyr Lys Ile Glu Gly Asp Glu Glu Met His Cys Ser Asp Asp Gly
 420 425 430
 Phe Trp Ser Lys Glu Lys Pro Lys Cys Val Glu Ile Ser Cys Lys Ser
 435 440 445
 Pro Asp Val Ile Asn Gly Ser Pro Ile Ser Gln Lys Ile Ile Tyr Lys
 450 455 460
 Glu Asn Glu Arg Phe Gln Tyr Lys Cys Asn Met Gly Tyr Glu Tyr Ser
 465 470 475 480
 Glu Arg Gly Asp Ala Val Cys Thr Glu Ser Gly Trp Arg Pro Leu Pro
 485 490 495
 Ser Cys Glu Glu Lys Ser Cys Asp Asn Pro Tyr Ile Pro Asn Gly Asp
 500 505 510
 Tyr Ser Pro Leu Arg Ile Lys His Arg Thr Gly Asp Glu Ile Thr Tyr
 515 520 525
 Gln Cys Arg Asn Gly Phe Tyr Pro Ala Thr Arg Gly Asn Thr Ala Lys
 530 535 540
 Cys Thr Ser Thr Gly Trp Ile Pro Ala Pro Arg Cys Thr
 545 550 555

<210> SEQ ID NO 10
 <211> LENGTH: 557
 <212> TYPE: PRT
 <213> ORGANISM: Murine
 <220> FEATURE:
 <221> NAME/KEY: VARIANT
 <222> LOCATION: (252)..(253)
 <223> OTHER INFORMATION: Xaa = Unknown or Other
 <220> FEATURE:
 <221> NAME/KEY: VARIANT
 <222> LOCATION: (252)..(254)
 <223> OTHER INFORMATION: Optional Linker

<400> SEQUENCE: 10

Ile Ser Cys Gly Ser Pro Pro Pro Ile Leu Asn Gly Arg Ile Ser Tyr
 1 5 10 15
 Tyr Ser Thr Pro Ile Ala Val Gly Thr Val Ile Arg Tyr Ser Cys Ser
 20 25 30
 Gly Thr Phe Arg Leu Ile Gly Glu Lys Ser Leu Leu Cys Ile Thr Lys
 35 40 45
 Asp Lys Val Asp Gly Thr Trp Asp Lys Pro Ala Pro Lys Cys Glu Tyr
 50 55 60
 Phe Asn Lys Tyr Ser Ser Cys Pro Glu Pro Ile Val Pro Gly Gly Tyr
 65 70 75 80
 Lys Ile Arg Gly Ser Thr Pro Tyr Arg His Gly Asp Ser Val Thr Phe
 85 90 95
 Ala Cys Lys Thr Asn Phe Ser Met Asn Gly Asn Lys Ser Val Trp Cys
 100 105 110
 Gln Ala Asn Asn Met Trp Gly Pro Thr Arg Leu Pro Thr Cys Val Ser
 115 120 125
 Val Phe Pro Leu Glu Cys Pro Ala Leu Pro Met Ile His Asn Gly His
 130 135 140

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| | | | | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| His | Thr | Ser | Glu | Asn | Val | Gly | Ser | Ile | Ala | Pro | Gly | Leu | Ser | Val | Thr | 145 | 150 | 155 | 160 |
| Tyr | Ser | Cys | Glu | Ser | Gly | Tyr | Leu | Leu | Val | Gly | Glu | Lys | Ile | Ile | Asn | 165 | 170 | 175 | |
| Cys | Leu | Ser | Ser | Gly | Lys | Trp | Ser | Ala | Val | Pro | Pro | Thr | Cys | Glu | Glu | 180 | 185 | 190 | |
| Ala | Arg | Cys | Lys | Ser | Leu | Gly | Arg | Phe | Pro | Asn | Gly | Lys | Val | Lys | Glu | 195 | 200 | 205 | |
| Pro | Pro | Ile | Leu | Arg | Val | Gly | Val | Thr | Ala | Asn | Phe | Phe | Cys | Asp | Glu | 210 | 215 | 220 | |
| Gly | Tyr | Arg | Leu | Gln | Gly | Pro | Pro | Ser | Ser | Arg | Cys | Val | Ile | Ala | Gly | 225 | 230 | 235 | 240 |
| Gln | Gly | Val | Ala | Trp | Thr | Lys | Met | Pro | Val | Cys | Xaa | Xaa | Xaa | Glu | Asp | 245 | 250 | 255 | |
| Cys | Asn | Glu | Leu | Pro | Pro | Arg | Arg | Asn | Thr | Glu | Ile | Leu | Thr | Gly | Ser | 260 | 265 | 270 | |
| Trp | Ser | Asp | Gln | Thr | Tyr | Pro | Glu | Gly | Thr | Gln | Ala | Ile | Tyr | Lys | Cys | 275 | 280 | 285 | |
| Arg | Pro | Gly | Tyr | Arg | Ser | Leu | Gly | Asn | Ile | Ile | Met | Val | Cys | Arg | Lys | 290 | 295 | 300 | |
| Gly | Glu | Trp | Val | Ala | Leu | Asn | Pro | Leu | Arg | Lys | Cys | Gln | Lys | Arg | Pro | 305 | 310 | 315 | 320 |
| Cys | Gly | His | Pro | Gly | Asp | Thr | Pro | Phe | Gly | Thr | Phe | Thr | Leu | Thr | Gly | 325 | 330 | 335 | |
| Gly | Asn | Val | Phe | Glu | Tyr | Gly | Val | Lys | Ala | Val | Tyr | Thr | Cys | Asn | Glu | 340 | 345 | 350 | |
| Gly | Tyr | Gln | Leu | Leu | Gly | Glu | Ile | Asn | Tyr | Arg | Glu | Cys | Asp | Thr | Asp | 355 | 360 | 365 | |
| Gly | Trp | Thr | Asn | Asp | Ile | Pro | Ile | Cys | Glu | Val | Val | Lys | Cys | Leu | Pro | 370 | 375 | 380 | |
| Val | Thr | Ala | Pro | Glu | Asn | Gly | Lys | Ile | Val | Ser | Ser | Ala | Met | Glu | Pro | 385 | 390 | 395 | 400 |
| Asp | Arg | Glu | Tyr | His | Phe | Gly | Gln | Ala | Val | Arg | Phe | Val | Cys | Asn | Ser | 405 | 410 | 415 | |
| Gly | Tyr | Lys | Ile | Glu | Gly | Asp | Glu | Glu | Met | His | Cys | Ser | Asp | Asp | Gly | 420 | 425 | 430 | |
| Phe | Trp | Ser | Lys | Glu | Lys | Pro | Lys | Cys | Val | Glu | Ile | Ser | Cys | Lys | Ser | 435 | 440 | 445 | |
| Pro | Asp | Val | Ile | Asn | Gly | Ser | Pro | Ile | Ser | Gln | Lys | Ile | Ile | Tyr | Lys | 450 | 455 | 460 | |
| Glu | Asn | Glu | Arg | Phe | Gln | Tyr | Lys | Cys | Asn | Met | Gly | Tyr | Glu | Tyr | Ser | 465 | 470 | 475 | 480 |
| Glu | Arg | Gly | Asp | Ala | Val | Cys | Thr | Glu | Ser | Gly | Trp | Arg | Pro | Leu | Pro | 485 | 490 | 495 | |
| Ser | Cys | Glu | Glu | Lys | Ser | Cys | Asp | Asn | Pro | Tyr | Ile | Pro | Asn | Gly | Asp | 500 | 505 | 510 | |
| Tyr | Ser | Pro | Leu | Arg | Ile | Lys | His | Arg | Thr | Gly | Asp | Glu | Ile | Thr | Tyr | 515 | 520 | 525 | |
| Gln | Cys | Arg | Asn | Gly | Phe | Tyr | Pro | Ala | Thr | Arg | Gly | Asn | Thr | Ala | Lys | 530 | 535 | 540 | |
| Cys | Thr | Ser | Thr | Gly | Trp | Ile | Pro | Ala | Pro | Arg | Cys | Thr | 545 | 550 | 555 | | | | |

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<210> SEQ ID NO 11
 <211> LENGTH: 21
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <221> NAME/KEY: VARIANT
 <222> LOCATION: (1)..(21)
 <223> OTHER INFORMATION: signal peptide

<400> SEQUENCE: 11

Met Pro Met Gly Ser Leu Gln Pro Leu Ala Thr Leu Tyr Leu Leu Gly
 1 5 10 15

Met Leu Val Ala Ser
 20

<210> SEQ ID NO 12
 <211> LENGTH: 72
 <212> TYPE: DNA
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (1)..(72)
 <223> OTHER INFORMATION: signal peptide

<400> SEQUENCE: 12

atgcccatgg ggtctctgca accgctggcc accttgacc tgctggggat gctggtcgct 60

tcttgctcga ga 72

<210> SEQ ID NO 13
 <211> LENGTH: 17
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <221> NAME/KEY: VARIANT
 <222> LOCATION: (1)..(17)
 <223> OTHER INFORMATION: signal peptide

<400> SEQUENCE: 13

Met Gly Ala Ala Gly Leu Leu Gly Val Phe Leu Ala Leu Val Ala Pro
 1 5 10 15

Gly

<210> SEQ ID NO 14
 <211> LENGTH: 60
 <212> TYPE: DNA
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (1)..(60)
 <223> OTHER INFORMATION: signal peptide

<400> SEQUENCE: 14

atgggcgcgcg cgggcctgct cggggttttc ttggctctcg tcgcaccggg ggtcctcggg 60

<210> SEQ ID NO 15
 <211> LENGTH: 1025
 <212> TYPE: PRT
 <213> ORGANISM: Mus musculus

<400> SEQUENCE: 15

Met Leu Thr Trp Phe Leu Phe Tyr Phe Ser Glu Ile Ser Cys Asp Pro
 1 5 10 15

Pro Pro Glu Val Lys Asn Ala Arg Lys Pro Tyr Tyr Ser Leu Pro Ile
 20 25 30

Val Pro Gly Thr Val Leu Arg Tyr Thr Cys Ser Pro Ser Tyr Arg Leu

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| 35 | | | | | 40 | | | | | 45 | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ile | Gly | Glu | Lys | Ala | Ile | Phe | Cys | Ile | Ser | Glu | Asn | Gln | Val | His | Ala |
| 50 | | | | | 55 | | | | | 60 | | | | | |
| Thr | Trp | Asp | Lys | Ala | Pro | Pro | Ile | Cys | Glu | Ser | Val | Asn | Lys | Thr | Ile |
| 65 | | | | | 70 | | | | | 75 | | | | | 80 |
| Ser | Cys | Ser | Asp | Pro | Ile | Val | Pro | Gly | Gly | Phe | Met | Asn | Lys | Gly | Ser |
| | | | | 85 | | | | | 90 | | | | | 95 | |
| Lys | Ala | Pro | Phe | Arg | His | Gly | Asp | Ser | Val | Thr | Phe | Thr | Cys | Lys | Ala |
| | | | 100 | | | | | 105 | | | | | 110 | | |
| Asn | Phe | Thr | Met | Lys | Gly | Ser | Lys | Thr | Val | Trp | Cys | Gln | Ala | Asn | Glu |
| | | 115 | | | | | 120 | | | | | 125 | | | |
| Met | Trp | Gly | Pro | Thr | Ala | Leu | Pro | Val | Cys | Glu | Ser | Asp | Phe | Pro | Leu |
| | | 130 | | | | 135 | | | | | | 140 | | | |
| Glu | Cys | Pro | Ser | Leu | Pro | Thr | Ile | His | Asn | Gly | His | His | Thr | Gly | Gln |
| 145 | | | | | 150 | | | | | 155 | | | | | 160 |
| His | Val | Asp | Gln | Phe | Val | Ala | Gly | Leu | Ser | Val | Thr | Tyr | Ser | Cys | Glu |
| | | | 165 | | | | | 170 | | | | | | 175 | |
| Pro | Gly | Tyr | Leu | Leu | Thr | Gly | Lys | Lys | Thr | Ile | Lys | Cys | Leu | Ser | Ser |
| | | | 180 | | | | | 185 | | | | | 190 | | |
| Gly | Asp | Trp | Asp | Gly | Val | Ile | Pro | Thr | Cys | Lys | Glu | Ala | Gln | Cys | Glu |
| | | 195 | | | | | 200 | | | | | 205 | | | |
| His | Pro | Gly | Lys | Phe | Pro | Asn | Gly | Gln | Val | Lys | Glu | Pro | Leu | Ser | Leu |
| | | 210 | | | | 215 | | | | | 220 | | | | |
| Gln | Val | Gly | Thr | Thr | Val | Tyr | Phe | Ser | Cys | Asn | Glu | Gly | Tyr | Gln | Leu |
| 225 | | | | | 230 | | | | | 235 | | | | | 240 |
| Gln | Gly | Gln | Pro | Ser | Ser | Gln | Cys | Val | Ile | Val | Glu | Gln | Lys | Ala | Ile |
| | | | 245 | | | | | | 250 | | | | | 255 | |
| Trp | Thr | Lys | Lys | Pro | Val | Cys | Lys | Glu | Ile | Leu | Cys | Pro | Pro | Pro | Pro |
| | | | 260 | | | | | 265 | | | | | 270 | | |
| Pro | Val | Arg | Asn | Gly | Ser | His | Thr | Gly | Ser | Phe | Ser | Glu | Asn | Val | Pro |
| | | 275 | | | | 280 | | | | | | 285 | | | |
| Tyr | Gly | Ser | Thr | Val | Thr | Tyr | Thr | Cys | Asp | Pro | Ser | Pro | Glu | Lys | Gly |
| | | 290 | | | | 295 | | | | | 300 | | | | |
| Val | Ser | Phe | Thr | Leu | Ile | Gly | Glu | Lys | Thr | Ile | Asn | Cys | Thr | Thr | Gly |
| 305 | | | | | 310 | | | | | 315 | | | | | 320 |
| Ser | Gln | Lys | Thr | Gly | Ile | Trp | Ser | Gly | Pro | Ala | Pro | Tyr | Cys | Val | Leu |
| | | | 325 | | | | | | 330 | | | | | 335 | |
| Ser | Thr | Ser | Ala | Val | Leu | Cys | Leu | Gln | Pro | Lys | Ile | Lys | Arg | Gly | Gln |
| | | | 340 | | | | | 345 | | | | | 350 | | |
| Ile | Leu | Ser | Ile | Leu | Lys | Asp | Ser | Tyr | Ser | Tyr | Asn | Asp | Thr | Val | Ala |
| | | 355 | | | | | 360 | | | | | 365 | | | |
| Phe | Ser | Cys | Glu | Pro | Gly | Phe | Thr | Leu | Lys | Gly | Asn | Arg | Ser | Ile | Arg |
| | | 370 | | | | 375 | | | | | 380 | | | | |
| Cys | Asn | Ala | His | Gly | Thr | Trp | Glu | Pro | Pro | Val | Pro | Val | Cys | Glu | Lys |
| 385 | | | | | 390 | | | | | 395 | | | | | 400 |
| Gly | Cys | Gln | Ala | Pro | Pro | Lys | Ile | Ile | Asn | Gly | Gln | Lys | Glu | Asp | Ser |
| | | | 405 | | | | | | 410 | | | | | 415 | |
| Tyr | Leu | Leu | Asn | Phe | Asp | Pro | Gly | Thr | Ser | Ile | Arg | Tyr | Ser | Cys | Asp |
| | | | 420 | | | | | 425 | | | | | 430 | | |
| Pro | Gly | Tyr | Leu | Leu | Val | Gly | Glu | Asp | Thr | Ile | His | Cys | Thr | Pro | Glu |
| | | 435 | | | | | 440 | | | | | 445 | | | |
| Gly | Lys | Trp | Thr | Pro | Ile | Thr | Pro | Gln | Cys | Thr | Val | Ala | Glu | Cys | Lys |
| | | 450 | | | | 455 | | | | | 460 | | | | |

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| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Val | Gly | Pro | His | Leu | Phe | Lys | Arg | Pro | Gln | Asn | Gln | Phe | Ile | Arg |
| 465 | | | | | 470 | | | | | 475 | | | | | 480 |
| Thr | Ala | Val | Asn | Ser | Ser | Cys | Asp | Glu | Gly | Phe | Gln | Leu | Ser | Glu | Ser |
| | | | | 485 | | | | | 490 | | | | | 495 | |
| Ala | Tyr | Gln | Leu | Cys | Gln | Gly | Thr | Ile | Pro | Trp | Phe | Ile | Glu | Ile | Arg |
| | | | 500 | | | | | 505 | | | | | 510 | | |
| Leu | Cys | Lys | Glu | Ile | Thr | Cys | Pro | Pro | Pro | Pro | Val | Ile | His | Asn | Gly |
| | | 515 | | | | | 520 | | | | | 525 | | | |
| Thr | His | Thr | Trp | Ser | Ser | Ser | Glu | Asp | Val | Pro | Tyr | Gly | Thr | Val | Val |
| | 530 | | | | | 535 | | | | | 540 | | | | |
| Thr | Tyr | Met | Cys | Tyr | Pro | Gly | Pro | Glu | Glu | Gly | Val | Lys | Phe | Lys | Leu |
| 545 | | | | | 550 | | | | | 555 | | | | | 560 |
| Ile | Gly | Glu | Gln | Thr | Ile | His | Cys | Thr | Ser | Asp | Ser | Arg | Gly | Arg | Gly |
| | | | | 565 | | | | | 570 | | | | | 575 | |
| Ser | Trp | Ser | Ser | Pro | Ala | Pro | Leu | Cys | Lys | Leu | Ser | Leu | Pro | Ala | Val |
| | | | 580 | | | | | 585 | | | | | 590 | | |
| Gln | Cys | Thr | Asp | Val | His | Val | Glu | Asn | Gly | Val | Lys | Leu | Thr | Asp | Asn |
| | | 595 | | | | | 600 | | | | | 605 | | | |
| Lys | Ala | Pro | Tyr | Phe | Tyr | Asn | Asp | Ser | Val | Met | Phe | Lys | Cys | Asp | Asp |
| | 610 | | | | | 615 | | | | | 620 | | | | |
| Gly | Tyr | Ile | Leu | Ser | Gly | Ser | Ser | Gln | Ile | Arg | Cys | Lys | Ala | Asn | Asn |
| 625 | | | | | 630 | | | | | 635 | | | | | 640 |
| Thr | Trp | Asp | Pro | Glu | Lys | Pro | Leu | Cys | Lys | Lys | Glu | Gly | Cys | Glu | Pro |
| | | | | 645 | | | | | 650 | | | | | 655 | |
| Met | Arg | Val | His | Gly | Leu | Pro | Asp | Asp | Ser | His | Ile | Lys | Leu | Val | Lys |
| | | | 660 | | | | | 665 | | | | | 670 | | |
| Arg | Thr | Cys | Gln | Asn | Gly | Tyr | Gln | Leu | Thr | Gly | Tyr | Thr | Tyr | Glu | Lys |
| | | 675 | | | | | 680 | | | | | 685 | | | |
| Cys | Gln | Asn | Ala | Glu | Asn | Gly | Thr | Trp | Phe | Lys | Lys | Ile | Glu | Val | Cys |
| | 690 | | | | | 695 | | | | | 700 | | | | |
| Thr | Val | Ile | Leu | Cys | Gln | Pro | Pro | Pro | Lys | Ile | Ala | Asn | Gly | Gly | His |
| 705 | | | | | 710 | | | | | 715 | | | | | 720 |
| Thr | Gly | Met | Met | Ala | Lys | His | Phe | Leu | Tyr | Gly | Asn | Glu | Val | Ser | Tyr |
| | | | | 725 | | | | | 730 | | | | | 735 | |
| Glu | Cys | Asp | Glu | Gly | Phe | Tyr | Leu | Leu | Gly | Glu | Lys | Ser | Leu | Gln | Cys |
| | | | 740 | | | | | 745 | | | | | 750 | | |
| Val | Asn | Asp | Ser | Lys | Gly | His | Gly | Ser | Trp | Ser | Gly | Pro | Pro | Pro | Gln |
| | | 755 | | | | | 760 | | | | | 765 | | | |
| Cys | Leu | Gln | Ser | Ser | Pro | Leu | Thr | His | Cys | Pro | Asp | Pro | Glu | Val | Lys |
| | 770 | | | | | 775 | | | | | 780 | | | | |
| His | Gly | Tyr | Lys | Leu | Asn | Lys | Thr | His | Ser | Ala | Phe | Ser | His | Asn | Asp |
| 785 | | | | | 790 | | | | | 795 | | | | | 800 |
| Ile | Val | His | Phe | Val | Cys | Asn | Gln | Gly | Phe | Ile | Met | Asn | Gly | Ser | His |
| | | | | 805 | | | | | 810 | | | | | 815 | |
| Leu | Ile | Arg | Cys | His | Thr | Asn | Asn | Thr | Trp | Leu | Pro | Gly | Val | Pro | Thr |
| | | | 820 | | | | | 825 | | | | | 830 | | |
| Cys | Ile | Arg | Lys | Ala | Ser | Leu | Gly | Cys | Gln | Ser | Pro | Ser | Thr | Ile | Pro |
| | | 835 | | | | | 840 | | | | | 845 | | | |
| Asn | Gly | Asn | His | Thr | Gly | Gly | Ser | Ile | Ala | Arg | Phe | Pro | Pro | Gly | Met |
| | 850 | | | | | 855 | | | | | 860 | | | | |
| Ser | Val | Met | Tyr | Ser | Cys | Tyr | Gln | Gly | Phe | Leu | Met | Ala | Gly | Glu | Ala |
| 865 | | | | | 870 | | | | | 875 | | | | | 880 |

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Pro Val Tyr Lys Glu Asn Glu Arg Tyr His Tyr Lys Cys Lys His Gly
 225 230 235 240
 Tyr Val Pro Lys Glu Arg Gly Asp Ala Val Cys Thr Gly Ser Gly Trp
 245 250 255
 Ser Ser Gln Pro Phe Cys Glu Glu Lys Arg Cys Ser Pro Pro Tyr Ile
 260 265 270
 Leu Asn Gly Ile Tyr Thr Pro His Arg Ile Ile His Arg Ser Asp Asp
 275 280 285
 Glu Ile Arg Tyr Glu Cys Asn Tyr Gly Phe Tyr Pro Val Thr Gly Ser
 290 295 300
 Thr Val Ser Lys Cys Thr Pro Thr Gly Trp Ile Pro Val Pro Arg Cys
 305 310 315 320
 Thr Leu Lys Pro Cys Glu Phe Pro Gln Phe Lys Tyr Gly Arg Leu Tyr
 325 330 335
 Tyr Glu Glu Ser Leu Arg Pro Asn Phe Pro Val Ser Ile Gly Asn Lys
 340 345 350
 Tyr Ser Tyr Lys Cys Asp Asn Gly Phe Ser Pro Pro Ser Gly Tyr Ser
 355 360 365
 Trp Asp Tyr Leu Arg Cys Thr Ala Gln Gly Trp Glu Pro Glu Val Pro
 370 375 380
 Cys Val Arg Lys Cys Val Phe His Tyr Val Glu Asn Gly Asp Ser Ala
 385 390 395 400
 Tyr Trp Glu Lys Val Tyr Val Gln Gly Gln Ser Leu Lys Val Gln Cys
 405 410 415
 Tyr Asn Gly Tyr Ser Leu Gln Asn Gly Gln Asp Thr Met Thr Cys Thr
 420 425 430
 Glu Asn Gly Trp Ser Pro Pro Pro Lys Cys Ile Arg Ile Lys Thr Cys
 435 440 445
 Ser Ala Ser Asp Ile His Ile Asp Asn Gly Phe Leu Ser Glu Ser Ser
 450 455 460
 Ser Ile Tyr Ala Leu Asn Arg Glu Thr Ser Tyr Arg Cys Lys Gln Gly
 465 470 475 480
 Tyr Val Thr Asn Thr Gly Glu Ile Ser Gly Ser Ile Thr Cys Leu Gln
 485 490 495
 Asn Gly Trp Ser Pro Gln Pro Ser Cys Ile Lys Ser Cys Asp Met Pro
 500 505 510
 Val Phe Glu Asn Ser Ile Thr Lys Asn Thr Arg Thr Trp Phe Lys Leu
 515 520 525
 Asn Asp Lys Leu Asp Tyr Glu Cys Leu Val Gly Phe Glu Asn Glu Tyr
 530 535 540
 Lys His Thr Lys Gly Ser Ile Thr Cys Thr Tyr Tyr Gly Trp Ser Asp
 545 550 555 560
 Thr Pro Ser Cys Tyr Glu Arg Glu Cys Ser Val Pro Thr Leu Asp Arg
 565 570 575
 Lys Leu Val Val Ser Pro Arg Lys Glu Lys Tyr Arg Val Gly Asp Leu
 580 585 590
 Leu Glu Phe Ser Cys His Ser Gly His Arg Val Gly Pro Asp Ser Val
 595 600 605
 Gln Cys Tyr His Phe Gly Trp Ser Pro Gly Phe Pro Thr Cys Lys Gly
 610 615 620
 Gln Val Ala Ser Cys Ala Pro Pro Leu Glu Ile Leu Asn Gly Glu Ile
 625 630 635 640

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| | | | | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|------|------|------|-----|
| Asn | Gly | Ala | Lys | Lys | Val | Glu | Tyr | Ser | His | Gly | Glu | Val | Val | Lys | Tyr | 645 | 650 | 655 | |
| Asp | Cys | Lys | Pro | Arg | Phe | Leu | Leu | Lys | Gly | Pro | Asn | Lys | Ile | Gln | Cys | 660 | 665 | 670 | |
| Val | Asp | Gly | Asn | Trp | Thr | Thr | Leu | Pro | Val | Cys | Ile | Glu | Glu | Glu | Arg | 675 | 680 | 685 | |
| Thr | Cys | Gly | Asp | Ile | Pro | Glu | Leu | Glu | His | Gly | Ser | Ala | Lys | Cys | Ser | 690 | 695 | 700 | |
| Val | Pro | Pro | Tyr | His | His | Gly | Asp | Ser | Val | Glu | Phe | Ile | Cys | Glu | Glu | 705 | 710 | 715 | 720 |
| Asn | Phe | Thr | Met | Ile | Gly | His | Gly | Ser | Val | Ser | Cys | Ile | Ser | Gly | Lys | 725 | 730 | 735 | |
| Trp | Thr | Gln | Leu | Pro | Lys | Cys | Val | Ala | Thr | Asp | Gln | Leu | Glu | Lys | Cys | 740 | 745 | 750 | |
| Arg | Val | Leu | Lys | Ser | Thr | Gly | Ile | Glu | Ala | Ile | Lys | Pro | Lys | Leu | Thr | 755 | 760 | 765 | |
| Glu | Phe | Thr | His | Asn | Ser | Thr | Met | Asp | Tyr | Lys | Cys | Arg | Asp | Lys | Gln | 770 | 775 | 780 | |
| Glu | Tyr | Glu | Arg | Ser | Ile | Cys | Ile | Asn | Gly | Lys | Trp | Asp | Pro | Glu | Pro | 785 | 790 | 795 | 800 |
| Asn | Cys | Thr | Ser | Lys | Thr | Ser | Cys | Pro | Pro | Pro | Pro | Gln | Ile | Pro | Asn | 805 | 810 | 815 | |
| Thr | Gln | Val | Ile | Glu | Thr | Thr | Val | Lys | Tyr | Leu | Asp | Gly | Glu | Lys | Leu | 820 | 825 | 830 | |
| Ser | Val | Leu | Cys | Gln | Asp | Asn | Tyr | Leu | Thr | Gln | Asp | Ser | Glu | Glu | Met | 835 | 840 | 845 | |
| Val | Cys | Lys | Asp | Gly | Arg | Trp | Gln | Ser | Leu | Pro | Arg | Cys | Ile | Glu | Lys | 850 | 855 | 860 | |
| Ile | Pro | Cys | Ser | Gln | Pro | Pro | Thr | Ile | Glu | His | Gly | Ser | Ile | Asn | Leu | 865 | 870 | 875 | 880 |
| Pro | Arg | Ser | Ser | Glu | Glu | Arg | Arg | Asp | Ser | Ile | Glu | Ser | Ser | Ser | His | 885 | 890 | 895 | |
| Glu | His | Gly | Thr | Thr | Phe | Ser | Tyr | Val | Cys | Asp | Asp | Gly | Phe | Arg | Ile | 900 | 905 | 910 | |
| Pro | Glu | Glu | Asn | Arg | Ile | Thr | Cys | Tyr | Met | Gly | Lys | Trp | Ser | Thr | Pro | 915 | 920 | 925 | |
| Pro | Arg | Cys | Val | Gly | Leu | Pro | Cys | Gly | Pro | Pro | Pro | Ser | Ile | Pro | Leu | 930 | 935 | 940 | |
| Gly | Thr | Val | Ser | Leu | Glu | Leu | Glu | Ser | Tyr | Gln | His | Gly | Glu | Glu | Val | 945 | 950 | 955 | 960 |
| Thr | Tyr | His | Cys | Ser | Thr | Gly | Phe | Gly | Ile | Asp | Gly | Pro | Ala | Phe | Ile | 965 | 970 | 975 | |
| Ile | Cys | Glu | Gly | Gly | Lys | Trp | Ser | Asp | Pro | Pro | Lys | Cys | Ile | Lys | Thr | 980 | 985 | 990 | |
| Asp | Cys | Asp | Val | Leu | Pro | Thr | Val | Lys | Asn | Ala | Ile | Ile | Arg | Gly | Lys | 995 | 1000 | 1005 | |
| Ser | Lys | Lys | Ser | Tyr | Arg | Thr | Gly | Glu | Gln | Val | Thr | Phe | Arg | Cys | 1010 | 1015 | 1020 | | |
| Gln | Ser | Pro | Tyr | Gln | Met | Asn | Gly | Ser | Asp | Thr | Val | Thr | Cys | Val | 1025 | 1030 | 1035 | | |
| Asn | Ser | Arg | Trp | Ile | Gly | Gln | Pro | Val | Cys | Lys | Asp | Asn | Ser | Cys | 1040 | 1045 | 1050 | | |
| Val | Asp | Pro | Pro | His | Val | Pro | Asn | Ala | Thr | Ile | Val | Thr | Arg | Thr | | | | | |

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| 1055 | 1060 | 1065 |
|---|------|------|
| Lys Asn Lys Tyr Leu His Gly Asp Arg Val Arg Tyr Glu Cys Asn 1070 | 1075 | 1080 |
| Lys Pro Leu Glu Leu Phe Gly Gln Val Glu Val Met Cys Glu Asn 1085 | 1090 | 1095 |
| Gly Ile Trp Thr Glu Lys Pro Lys Cys Arg Gly Leu Phe Asp Leu 1100 | 1105 | 1110 |
| Ser Leu Lys Pro Ser Asn Val Phe Ser Leu Asp Ser Thr Gly Lys 1115 | 1120 | 1125 |
| Cys Gly Pro Pro Pro Pro Ile Asp Asn Gly Asp Ile Thr Ser Leu 1130 | 1135 | 1140 |
| Ser Leu Pro Val Tyr Glu Pro Leu Ser Ser Val Glu Tyr Gln Cys 1145 | 1150 | 1155 |
| Gln Lys Tyr Tyr Leu Leu Lys Gly Lys Lys Thr Ile Thr Cys Thr 1160 | 1165 | 1170 |
| Asn Gly Lys Trp Ser Glu Pro Pro Thr Cys Leu His Ala Cys Val 1175 | 1180 | 1185 |
| Ile Pro Glu Asn Ile Met Glu Ser His Asn Ile Ile Leu Lys Trp 1190 | 1195 | 1200 |
| Arg His Thr Glu Lys Ile Tyr Ser His Ser Gly Glu Asp Ile Glu 1205 | 1210 | 1215 |
| Phe Gly Cys Lys Tyr Gly Tyr Tyr Lys Ala Arg Asp Ser Pro Pro 1220 | 1225 | 1230 |
| Phe Arg Thr Lys Cys Ile Asn Gly Thr Ile Asn Tyr Pro Thr Cys 1235 | 1240 | 1245 |

Val

<210> SEQ ID NO 17

<211> LENGTH: 559

<212> TYPE: PRT

<213> ORGANISM: Mus musculus

<400> SEQUENCE: 17

| |
|--|
| Ile Ser Cys Asp Pro Pro Pro Glu Val Lys Asn Ala Arg Lys Pro Tyr 1 5 10 15 |
| Tyr Ser Leu Pro Ile Val Pro Gly Thr Val Leu Arg Tyr Thr Cys Ser 20 25 30 |
| Pro Ser Tyr Arg Leu Ile Gly Glu Lys Ala Ile Phe Cys Ile Ser Glu 35 40 45 |
| Asn Gln Val His Ala Thr Trp Asp Lys Ala Pro Pro Ile Cys Glu Ser 50 55 60 |
| Val Asn Lys Thr Ile Ser Cys Ser Asp Pro Ile Val Pro Gly Gly Phe 65 70 75 80 |
| Met Asn Lys Gly Ser Lys Ala Pro Phe Arg His Gly Asp Ser Val Thr 85 90 95 |
| Phe Thr Cys Lys Ala Asn Phe Thr Met Lys Gly Ser Lys Thr Val Trp 100 105 110 |
| Cys Gln Ala Asn Glu Met Trp Gly Pro Thr Ala Leu Pro Val Cys Glu 115 120 125 |
| Ser Asp Phe Pro Leu Glu Cys Pro Ser Leu Pro Thr Ile His Asn Gly 130 135 140 |
| His His Thr Gly Gln His Val Asp Gln Phe Val Ala Gly Leu Ser Val 145 150 155 160 |
| Thr Tyr Ser Cys Glu Pro Gly Tyr Leu Leu Thr Gly Lys Lys Thr Ile |

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| 165 | | | | | 170 | | | | | 175 | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Lys | Cys | Leu | Ser | Ser | Gly | Asp | Trp | Asp | Gly | Val | Ile | Pro | Thr | Cys | Lys |
| | | | 180 | | | | | 185 | | | | | | 190 | |
| Glu | Ala | Gln | Cys | Glu | His | Pro | Gly | Lys | Phe | Pro | Asn | Gly | Gln | Val | Lys |
| | | 195 | | | | | 200 | | | | | 205 | | | |
| Glu | Pro | Leu | Ser | Leu | Gln | Val | Gly | Thr | Thr | Val | Tyr | Phe | Ser | Cys | Asn |
| | 210 | | | | | 215 | | | | | 220 | | | | |
| Glu | Gly | Tyr | Gln | Leu | Gln | Gly | Gln | Pro | Ser | Ser | Gln | Cys | Val | Ile | Val |
| 225 | | | | | 230 | | | | | 235 | | | | | 240 |
| Glu | Gln | Lys | Ala | Ile | Trp | Thr | Lys | Lys | Pro | Val | Cys | Lys | Glu | Ile | Leu |
| | | | | 245 | | | | | 250 | | | | | 255 | |
| Glu | Asp | Cys | Lys | Gly | Pro | Pro | Pro | Arg | Glu | Asn | Ser | Glu | Ile | Leu | Ser |
| | | | 260 | | | | | 265 | | | | | 270 | | |
| Gly | Ser | Trp | Ser | Glu | Gln | Leu | Tyr | Pro | Glu | Gly | Thr | Gln | Ala | Thr | Tyr |
| | | 275 | | | | | 280 | | | | | 285 | | | |
| Lys | Cys | Arg | Pro | Gly | Tyr | Arg | Thr | Leu | Gly | Thr | Ile | Val | Lys | Val | Cys |
| | 290 | | | | | 295 | | | | | 300 | | | | |
| Lys | Asn | Gly | Lys | Trp | Val | Ala | Ser | Asn | Pro | Ser | Arg | Ile | Cys | Arg | Lys |
| 305 | | | | | 310 | | | | | 315 | | | | | 320 |
| Lys | Pro | Cys | Gly | His | Pro | Gly | Asp | Thr | Pro | Phe | Gly | Ser | Phe | Arg | Leu |
| | | | | 325 | | | | | 330 | | | | | 335 | |
| Ala | Val | Gly | Ser | Gln | Phe | Glu | Phe | Gly | Ala | Lys | Val | Val | Tyr | Thr | Cys |
| | | | 340 | | | | | 345 | | | | | 350 | | |
| Asp | Asp | Gly | Tyr | Gln | Leu | Leu | Gly | Glu | Ile | Asp | Tyr | Arg | Glu | Cys | Gly |
| | | 355 | | | | | 360 | | | | | 365 | | | |
| Ala | Asp | Gly | Trp | Ile | Asn | Asp | Ile | Pro | Leu | Cys | Glu | Val | Val | Lys | Cys |
| | 370 | | | | | 375 | | | | | 380 | | | | |
| Leu | Pro | Val | Thr | Glu | Leu | Glu | Asn | Gly | Arg | Ile | Val | Ser | Gly | Ala | Ala |
| 385 | | | | | 390 | | | | | 395 | | | | | 400 |
| Glu | Thr | Asp | Gln | Glu | Tyr | Tyr | Phe | Gly | Gln | Val | Val | Arg | Phe | Glu | Cys |
| | | | 405 | | | | | | 410 | | | | | 415 | |
| Asn | Ser | Gly | Phe | Lys | Ile | Glu | Gly | His | Lys | Glu | Ile | His | Cys | Ser | Glu |
| | | | 420 | | | | | 425 | | | | | 430 | | |
| Asn | Gly | Leu | Trp | Ser | Asn | Glu | Lys | Pro | Arg | Cys | Val | Glu | Ile | Leu | Cys |
| | | 435 | | | | | 440 | | | | | 445 | | | |
| Thr | Pro | Pro | Arg | Val | Glu | Asn | Gly | Asp | Gly | Ile | Asn | Val | Lys | Pro | Val |
| | 450 | | | | | 455 | | | | | 460 | | | | |
| Tyr | Lys | Glu | Asn | Glu | Arg | Tyr | His | Tyr | Lys | Cys | Lys | His | Gly | Tyr | Val |
| 465 | | | | | 470 | | | | | 475 | | | | | 480 |
| Pro | Lys | Glu | Arg | Gly | Asp | Ala | Val | Cys | Thr | Gly | Ser | Gly | Trp | Ser | Ser |
| | | | | 485 | | | | | 490 | | | | | 495 | |
| Gln | Pro | Phe | Cys | Glu | Glu | Lys | Arg | Cys | Ser | Pro | Pro | Tyr | Ile | Leu | Asn |
| | | | 500 | | | | | 505 | | | | | 510 | | |
| Gly | Ile | Tyr | Thr | Pro | His | Arg | Ile | Ile | His | Arg | Ser | Asp | Asp | Glu | Ile |
| | | 515 | | | | | 520 | | | | | 525 | | | |
| Arg | Tyr | Glu | Cys | Asn | Tyr | Gly | Phe | Tyr | Pro | Val | Thr | Gly | Ser | Thr | Val |
| | 530 | | | | | 535 | | | | | 540 | | | | |
| Ser | Lys | Cys | Thr | Pro | Thr | Gly | Trp | Ile | Pro | Val | Pro | Arg | Cys | Thr | |
| 545 | | | | | 550 | | | | | 555 | | | | | |

<210> SEQ ID NO 18

<211> LENGTH: 1750

<212> TYPE: DNA

<213> ORGANISM: Mus musculus

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<400> SEQUENCE: 18

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tccgtgctag cgatttcttg tgaccctcct cctgaagtca aaaatgctcg gaaaccctat 120
tattctcttc ccatagtcc tggaactggt ctgaggtaca cttgttcacc tagctaccgc 180
ctcattggag aaaaggctat cttttgtata agtgaaaatc aagtgcacgc cacctgggat 240
aaagctcctc ctatatgtga atctgtgaat aaaaccattt cttgctcaga tcccatagta 300
ccagggggat tcatgaataa aggatctaag gcaccattca gacatgggta ttctgtgaca 360
ttacctgta aagccaactt caccatgaaa ggaagcaaaa ctgtctgggtg ccaggcaaat 420
gaaatgtggg gaccaacagc tctgccagtc tgtgagagtg atttcctct ggagtgccca 480
tcacttccaa cgattcataa tggacaccac acaggacagc atggtgacca gtttgttgcg 540
gggttgctg tgacatacag ttgtgaacct ggctatctgc tcaactggaaa aaagacaatt 600
aagtgcttat cttcaggaga ctgggatggt gtcacccga catgcaaaga ggcccagtgt 660
gaacatccag gaaagtttcc caatgggcag gtaaaggaac ctctgagcct tcaggttggc 720
acaactgtgt acttctcctg taatgaaggg taccaattac aaggacaacc ctctagtcag 780
tgtgtaattg ttgaacagaa agccatctgg actaagaagc cagtatgtaa agaaattctc 840
gaagattgta aaggtcctcc tccaagagaa aattcagaaa ttctctcagg ctctgtgtca 900
gaacaactat atccagaagg caccaggct acctacaaat gccgccttg ataccgaaca 960
cttggcacta ttgtaaaagt atgcaagaat ggaaaatggg tggcgtctaa cccatccagg 1020
atatgtcgga aaaagccttg tgggcatccc ggagacacac cctttgggtc ctttaggctg 1080
gcagttggat ctcaatttga gtttgggtgca aaggttgttt atacctgtga tgatgggtat 1140
caactattag gtgaaattga ttaccgtgaa tgtggtgcag atggctggat caatgatatt 1200
ccactatgtg aagttgtgaa gtgtctacct gtgacagAAC tcgagaatgg aagaattgtg 1260
agtgggtgcag cagaaacaga ccaggaatac tattttggac aggtgggtgcg gtttgaatgc 1320
aattcaggct tcaagattga aggacataag gaaattcatt gctcagaaaa tggcctttgg 1380
agcaatgaaa agccacgatg tgtggaaatt ctctgcacac caccgcgagt ggaaaatgga 1440
gatggtataa atgtgaaacc agtttacaag gagaatgaaa gataccacta taagtgtaag 1500
catggttatg tgcccaaaga aagaggggat gccgtctgca caggctctgg atggagttct 1560
cagcctttct gtgaagaaaa gagatgctca cctccttata ttctaaatgg tatctacaca 1620
cctcacagga ttatacacag aagtgatgat gaaatcagat atgaatgtaa ttatggcttc 1680
tatectgtaa ctggatcaac tgtttcaaag tgtacacca ctggctggat cctgttcca 1740
agatgtacct 1750

<210> SEQ ID NO 19

<211> LENGTH: 2676

<212> TYPE: DNA

<213> ORGANISM: Mus musculus

<400> SEQUENCE: 19

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gggatgctgg tegettcctg gctagegatt tcttgtgacc ctctcctga agtcaaaaat 120
gctcggaaac cctattatc tcttccata gttcctggaa ctgttctgag gtacacttgt 180
tcacctagct accgcctcat tggagaaaag gctatctttt gtataagtga aatcaagtg 240

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| | | | | | | |
|-------------|------------|------------|------------|------------|-------------|------|
| catgccacct | gggataaagc | tctcctata | tgtgaatctg | tgaataaaac | catttcttgc | 300 |
| tcagatocca | tagtaccagg | gggattcatg | aataaaggat | ctaaggcacc | attcagacat | 360 |
| ggtgattctg | tgacatttac | ctgtaaagcc | aacttcacca | tgaaaggaag | caaaactgtc | 420 |
| tggtgccagg | caaatgaaat | gtggggacca | acagctctgc | cagtctgtga | gagtgatttc | 480 |
| cctctggagt | gcccacact | tccaacgatt | cataatggac | accacacagg | acagcatggt | 540 |
| gaccagtttg | ttgcggggtt | gtctgtgaca | tacagttgtg | aacctggcta | tttgetcact | 600 |
| ggaaaaaaga | caattaagtg | cttatcttca | ggagactggg | atgggtgcat | cccagacatg | 660 |
| aaagaggccc | agtgtgaaca | tccaggaaag | tttcccaatg | ggcaggtaaa | ggaacctctg | 720 |
| agccttcagg | ttggcacaac | tgtgtacttc | tctgtaatg | aagggtacca | attacaagga | 780 |
| caaccctcta | gtcagtgtgt | aattgttgaa | cagaaagcca | tctggactaa | gaagccagta | 840 |
| tgtaaagaaa | ttctcgaaga | ttgtaaaggt | cctcctccaa | gagaaaattc | agaaattctc | 900 |
| tcaggctcgt | ggtcagaaca | actatatcca | gaaggcacc | aggctaccta | caaatgccgc | 960 |
| cctggatacc | gaacacttgg | cactattgta | aaagtatgca | agaatggaaa | atgggtggcg | 1020 |
| tctaaccocat | ccaggatatg | tcggaaaaag | ccttgtgggc | atcccggaga | cacacccttt | 1080 |
| gggtccttta | ggctggcagt | tggatctcaa | tttgagtttg | gtgcaaaggt | tgtttatacc | 1140 |
| tgtgatgatg | ggtatcaact | attaggtgaa | attgattacc | gtgaatgtgg | tgacagatggc | 1200 |
| tggtcaatg | atattccact | atgtgaagtt | gtgaagtgtc | tacctgtgac | agaactcgag | 1260 |
| aatggaagaa | ttgtgagtgg | tgacagcaga | acagaccagg | aatactatct | tgacaggtg | 1320 |
| gtgcggtttg | aatgcaattc | aggcttcaag | attgaaggac | ataaggaaat | tcattgctca | 1380 |
| gaaaatggcc | tttgagcaa | tgaaaagcca | cgatgtgtgg | aaattctctg | cacaccaccg | 1440 |
| cgagtggaaa | atggagatgg | tataaatgtg | aaaccagttt | acaaggagaa | tgaaagatac | 1500 |
| cactataagt | gtaagcatgg | ttatgtgcc | aaagaaagag | gggatgccgt | ctgcacaggc | 1560 |
| tctggatgga | gttctcagcc | tttctgtgaa | gaaaagagat | gctcacctcc | ttatattcta | 1620 |
| aatggtatct | acacacctca | caggattata | cacagaagtg | atgatgaaat | cagatatgaa | 1680 |
| tgtaattatg | gcttctatcc | tgtaactgga | tcaactgttt | caaagtgtac | accactggc | 1740 |
| tggtccctg | ttccaagatg | taccgaagat | tgtaaaggtc | ctcctccaag | agaaaattca | 1800 |
| gaaattctct | caggctcgtg | gtcagaacaa | ctatatccag | aaggcaccca | ggctacctac | 1860 |
| aaatgccgcc | ctggataccg | aacacttggc | actattgtaa | aagtatgcaa | gaatggaaaa | 1920 |
| tggttggcgt | ctaaccatc | caggatatgt | cgaaaaaagc | cttgtgggca | tcccggagac | 1980 |
| acacccttg | ggctcttag | gctggcagtt | ggatctcaat | ttgagtttgg | tgcaaagggt | 2040 |
| gtttatacct | gtgatgatgg | gtatcaacta | ttaggtgaaa | ttgattaccg | tgaatgtgg | 2100 |
| gcagatggct | ggatcaatga | tattccacta | tgtgaagttg | tgaagtgtct | acctgtgaca | 2160 |
| gaactcgaga | atggaagaat | tgtgagtgg | gcagcagaaa | cagaccagga | atactatctt | 2220 |
| ggacaggtgg | tgcggtttga | atgcaattca | ggcttcaaga | ttgaaggaca | taaggaaatt | 2280 |
| cattgctcag | aaaatggcct | ttggagcaat | gaaaagccac | gatgtgtgga | aattctctgc | 2340 |
| acaccaccgc | gagtggaaaa | tggagatgg | ataaatgtga | aaccagttta | caaggagaat | 2400 |
| gaaagatacc | actataagtg | taagcatgg | tatgtgcca | aagaaagagg | ggatgccgtc | 2460 |
| tgacacagct | ctggatggag | ttctcagcct | ttctgtgaag | aaaagagatg | ctcacctcct | 2520 |
| tatattctaa | atggtatcta | cacacctcac | aggattatac | acagaagtga | tgatgaaatc | 2580 |
| agatatgaat | gtaattatgg | cttctatcct | gtaactggat | caactgtttc | aaagtgtaca | 2640 |

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cccactggct ggatccctgt tccaagatgt acctaa 2676

<210> SEQ ID NO 20

<211> LENGTH: 2706

<212> TYPE: DNA

<213> ORGANISM: Mus musculus

<400> SEQUENCE: 20

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gggatgctgg tcgcttccgt gctagcgatt tcttgtagacc ctctctctga agtcaaaaat 120
gctcggaaac cctattatc tcttccata gttcctggaa ctggtctgag gtacacttgt 180
tcacctagct accgcctcat tggagaaaag gctatctttt gtataagtga aaatcaagtg 240
catgccacct gggataaagc tctcctata tgtgaatctg tgaataaaac catttcttgc 300
tcagatccca tagtaccagg gggattcatg aataaaggat ctaaggcacc attcagacat 360
ggtgattctg tgacatttac ctgtaaagcc aacttcacca tgaaggaag caaaactgtc 420
tggtgccagg caaatgaaat gtggggacca acagctctgc cagtctgtga gagtgatttc 480
cctctggagt gcccatcact tccaacgatt cataatggac accacacagg acagcatggt 540
gaccagtttg ttgctgggtt gtctgtgaca tacagttgtg aacctggcta tttgctcact 600
ggaaaaaaga caattaagtg cttatcttca ggagactggg atgggtgcat cccgacatgc 660
aaagaggccc agtgtgaaca tccaggaaaag tttcccaatg ggcaggtaa ggaacctctg 720
agccttcagg ttggcacaac tgtgtacttc tctgtaatg aagggtacca attacaagga 780
caaccctcta gtcagtgtgt aattgttgaa cagaaagcca tctggactaa gaagccagta 840
tgtaaagaaa ttctcggcgg aggtgggtcg ggtggcggcg gatctgaaga ttgtaaaggt 900
ctcctccaa gagaaaattc agaaattctc tcaggctcgt ggtcagaaca actatatcca 960
gaaggcacc aggtaccta caaatgccgc cctggatacc gaacacttgg cactattgta 1020
aaagtatgca agaatggaaa atgggtggcg tctaaccat ccaggatatg tcggaaaaag 1080
ccttggtggc atcccgaga cacaccctt gggtccttta ggctggcagt tggatctcaa 1140
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attgattacc gtgaatgtgg tgcagatggc tggatcaatg atattccact atgtgaagtt 1260
gtgaagtgtc tacctgtgac agaactcgag aatggaagaa ttgtgagtgg tgcagcagaa 1320
acagaccagg aatactattt tggacagggt gtgctggttg aatgcaattc aggcttcaag 1380
attgaaggac ataaggaat tcattgctca gaaaatggcc tttggagcaa tgaaaagcca 1440
cgatgtgtgg aaattctctg cacaccaccg cgagtggaaa atggagatgg tataaatgtg 1500
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aaagaaagag gggatgccgt ctgcacaggc tctggatgga gttctcagcc tttctgtgaa 1620
gaaaagagat gctcacctcc ttatattcta aatggtatct acacacctca caggattata 1680
cacagaagtg atgatgaaat cagatatgaa tgtaattatg gcttctatcc tgtaactgga 1740
tcaactgttt caaagtgtac acccactggc tggatccctg ttccaagatg taccgaagat 1800
tgtaaaggtc ctctccaag agaaaattca gaaattctct caggctcgtg gtcagaacaa 1860
ctatatccag aaggcacca ggctacctac aaatgccgcc ctggataccg aacacttggc 1920
actattgtaa aagtatgcaa gaatggaaaa tgggtggcgt ctaaccatc caggatatgt 1980
cggaaaaagc cttgtgggca tcccgagac acacccttg ggtcctttag gctggcagtt 2040

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ggatctcaat ttgagtttgg tgcaaagggt gtttatacct gtgatgatgg gtatcaacta 2100
ttaggtgaaa ttgattaccg tgaatgtggt gcagatggct ggatcaatga tattccacta 2160
tgtgaagttg tgaagtgtct acctgtgaca gaactcgaga atggaagaat tgtgagtgg 2220
gcagcagaaa cagaccagga atactatfff ggacaggtgg tgcggtttga atgcaattca 2280
ggcttcaaga ttgaaggaca taaggaaatt cattgtctag aaaatggcct ttggagcaat 2340
gaaaagccac gatgtgtgga aattctctgc acaccaccgc gagtggaaaa tggagatgg 2400
ataaatgtga aaccagttta caaggagaat gaaagatacc actataagtg taagcatgg 2460
tatgtgcccc aagaagagg ggatgccgct tgcacaggct ctggatggag ttctcagcct 2520
ttctgtgaag aaaagagatg ctcacctcct tatattctaa atggtatcta cacacctcac 2580
aggattatac acagaagtga tgatgaaatc agatatgaat gtaattatgg cttctatcct 2640
gtaactggat caactgtttc aaagtgtaca cccactggct ggatccctgt tccaagatgt 2700
acctaa 2706

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<210> SEQ ID NO 21

<211> LENGTH: 560

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 21

```

Ile Ser Cys Gly Ser Pro Pro Pro Ile Leu Asn Gly Arg Ile Ser Tyr
1          5          10          15

Tyr Ser Thr Pro Ile Ala Val Gly Thr Val Ile Arg Tyr Ser Cys Ser
          20          25          30

Gly Thr Phe Arg Leu Ile Gly Glu Lys Ser Leu Leu Cys Ile Thr Lys
          35          40          45

Asp Lys Val Asp Gly Thr Trp Asp Lys Pro Ala Pro Lys Cys Glu Tyr
          50          55          60

Phe Asn Lys Tyr Ser Ser Cys Pro Glu Pro Ile Val Pro Gly Gly Tyr
65          70          75          80

Lys Ile Arg Gly Ser Thr Pro Tyr Arg His Gly Asp Ser Val Thr Phe
          85          90          95

Ala Cys Lys Thr Asn Phe Ser Met Asn Gly Asn Lys Ser Val Trp Cys
100          105          110

Gln Ala Asn Asn Met Trp Gly Pro Thr Arg Leu Pro Thr Cys Val Ser
115          120          125

Val Phe Pro Leu Glu Cys Pro Ala Leu Pro Met Ile His Asn Gly His
130          135          140

His Thr Ser Glu Asn Val Gly Ser Ile Ala Pro Gly Leu Ser Val Thr
145          150          155          160

Tyr Ser Cys Glu Ser Gly Tyr Leu Leu Val Gly Glu Lys Ile Ile Asn
165          170          175

Cys Leu Ser Ser Gly Lys Trp Ser Ala Val Pro Pro Thr Cys Glu Glu
180          185          190

Ala Arg Cys Lys Ser Leu Gly Arg Phe Pro Asn Gly Lys Val Lys Glu
195          200          205

Pro Pro Ile Leu Arg Val Gly Val Thr Ala Asn Phe Phe Cys Asp Glu
210          215          220

Gly Tyr Arg Leu Gln Gly Pro Pro Ser Ser Arg Cys Val Ile Ala Gly
225          230          235          240

Gln Gly Val Ala Trp Thr Lys Met Pro Val Cys Glu Glu Ile Phe Glu
245          250          255

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Asp Cys Asn Glu Leu Pro Pro Arg Arg Asn Thr Glu Ile Leu Thr Gly
 260 265 270

Ser Trp Ser Asp Gln Thr Tyr Pro Glu Gly Thr Gln Ala Ile Tyr Lys
 275 280 285

Cys Arg Pro Gly Tyr Arg Ser Leu Gly Asn Val Ile Met Val Cys Arg
 290 295 300

Lys Gly Glu Trp Val Ala Leu Asn Pro Leu Arg Lys Cys Gln Lys Arg
 305 310 315 320

Pro Cys Gly His Pro Gly Asp Thr Pro Phe Gly Thr Phe Thr Leu Thr
 325 330 335

Gly Gly Asn Val Phe Glu Tyr Gly Val Lys Ala Val Tyr Thr Cys Asn
 340 345 350

Glu Gly Tyr Gln Leu Leu Gly Glu Ile Asn Tyr Arg Glu Cys Asp Thr
 355 360 365

Asp Gly Trp Thr Asn Asp Ile Pro Ile Cys Glu Val Val Lys Cys Leu
 370 375 380

Pro Val Thr Ala Pro Glu Asn Gly Lys Ile Val Ser Ser Ala Met Glu
 385 390 395 400

Pro Asp Arg Glu Tyr His Phe Gly Gln Ala Val Arg Phe Val Cys Asn
 405 410 415

Ser Gly Tyr Lys Ile Glu Gly Asp Glu Glu Met His Cys Ser Asp Asp
 420 425 430

Gly Phe Trp Ser Lys Glu Lys Pro Lys Cys Val Glu Ile Ser Cys Lys
 435 440 445

Ser Pro Asp Val Ile Asn Gly Ser Pro Ile Ser Gln Lys Ile Ile Tyr
 450 455 460

Lys Glu Asn Glu Arg Phe Gln Tyr Lys Cys Asn Met Gly Tyr Glu Tyr
 465 470 475 480

Ser Glu Arg Gly Asp Ala Val Cys Thr Glu Ser Gly Trp Arg Pro Leu
 485 490 495

Pro Ser Cys Glu Glu Lys Ser Cys Asp Asn Pro Tyr Ile Pro Asn Gly
 500 505 510

Asp Tyr Ser Pro Leu Arg Ile Lys His Arg Thr Gly Asp Glu Ile Thr
 515 520 525

Tyr Gln Cys Arg Asn Gly Phe Tyr Pro Ala Thr Arg Gly Asn Thr Ala
 530 535 540

Lys Cys Thr Ser Thr Gly Trp Ile Pro Ala Pro Arg Cys Thr Leu Lys
 545 550 555 560

<210> SEQ ID NO 22
 <211> LENGTH: 1755
 <212> TYPE: DNA
 <213> ORGANISM: Homo sapiens
 <400> SEQUENCE: 22

gccgccacca tgggagccgc tgggtctgctc ggcgtgttcc tcgccttggg ggcacctggc 60
 gtccctgggca tcagctgagg ttcccctcca ccaatcctga atggcagaat ctccattacc 120
 tccacaccaa tcgccgtcgg cactgtgatc agatacagct gttcaggac ttttcggctg 180
 atcggcgaga aaagcctcct ctgcattacc aaggataagg tcgatgggac atgggataaa 240
 ccagctccta agtgcgagta cttcaataag tatagttcat gtccagagcc cattgttcc 300
 ggtggctaca agattcgggg gagcacaccc tatcgccaag gtgactcagt gacctttgct 360
 tgtaaaacca acttctcaat gaacggtaat aagtcagtgt ggtgtcaggc caataatag 420

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tggggtccta cacgactccc cacctgtgtg tccgtgttcc ccttggaatg ccccgccctg 480
cccatgatcc ataatggaca ccacaccagc gagaatgtcg ggagtatcgc acctggattg 540
agtgtcacct actcatgcca gtctggctac ctgctttagt gtgaaaaaat tattaattgc 600
ttgtcctccg gcaaatggag tgccgttccc ccaacttgtg aagaggcccc gtgcaaatcc 660
ctcggccgct tcctaataag taaagttaaa gagcctccaa tcctcagagt gggggtgacc 720
gctaacttct tctgtgatga aggctaccgg ttgcagggac caccagtag ccgggtgtgc 780
atagctgggc agggagtggc ttggacaaaag atgcccgttt gtgaggaaat cttcgaagac 840
tgtaatgagc tgcccccaag acggaataca gagatcctca caggctcttg gtccgatcaa 900
acttatccag agggtagcca ggcaatttac aagtgcagac ctggatacag gagcctgggc 960
aatgtgatta tgggtgtgcc caagggggag tgggtggccc ttaatcctct ccggaagtgt 1020
cagaaaagac catgcggaca ccctggagat acacctttcg gtacctttac ccttaccggc 1080
ggcaatgtct tcgagtatgg cgtcaaggcc gtgtacactt gtaacgaggg ataccagctg 1140
ctgggggaaa taaactatcg tgagtgtgac actgacgggt ggactaacga catccccatt 1200
tgcgaggtgg tcaagtgcct tcctgtaacc gctcccgaat atggttaagat cgtatcttcc 1260
gcaatggagc ctgatcggga ataccacttt ggacaagccg ttcggttcgt atgtaattca 1320
gggtataaaa ttgagggcga tgaggagatg cactgcagtg atgacggctt ttggtcaaag 1380
gaaaagccaa agtgcgtaga gatcagttgt aagtctctcg acgttattaa cgggagtccc 1440
atcagtcaga agatcattta caaggaaaac gagaggttcc agtataaatg caatatggga 1500
tatgagtact ccgaaagagg ggacgccgtg tgcacagagt ccggatggcg acctttgcca 1560
tcttgtgaag aaaagtcttg tgacaacccc tatattccta acggagatta ctctcctctg 1620
cgcacaaagc accgaactgg ggacgagatc acttaccaat gtcgaaacgg cttctaccct 1680
gctaccagag gtaaacactgc caagtgtacc agcaccgggt ggattcccgc cccagatgc 1740
acacttaaat gataa 1755

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<210> SEQ ID NO 23

<211> LENGTH: 863

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 23

```

Ile Ser Cys Gly Ser Pro Pro Pro Ile Leu Asn Gly Arg Ile Ser Tyr
1           5           10           15
Tyr Ser Thr Pro Ile Ala Val Gly Thr Val Ile Arg Tyr Ser Cys Ser
20           25           30
Gly Thr Phe Arg Leu Ile Gly Glu Lys Ser Leu Leu Cys Ile Thr Lys
35           40           45
Asp Lys Val Asp Gly Thr Trp Asp Lys Pro Ala Pro Lys Cys Glu Tyr
50           55           60
Phe Asn Lys Tyr Ser Ser Cys Pro Glu Pro Ile Val Pro Gly Gly Tyr
65           70           75           80
Lys Ile Arg Gly Ser Thr Pro Tyr Arg His Gly Asp Ser Val Thr Phe
85           90           95
Ala Cys Lys Thr Asn Phe Ser Met Asn Gly Asn Lys Ser Val Trp Cys
100          105          110
Gln Ala Asn Asn Met Trp Gly Pro Thr Arg Leu Pro Thr Cys Val Ser
115          120          125

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| | | | |
|---|-----|-----|-----|
| 545 | 550 | 555 | 560 |
| Cys Asn Glu Leu Pro Pro Arg Arg Asn Thr Glu Ile Leu Thr Gly Ser | 565 | 570 | 575 |
| Trp Ser Asp Gln Thr Tyr Pro Glu Gly Thr Gln Ala Ile Tyr Lys Cys | 580 | 585 | 590 |
| Arg Pro Gly Tyr Arg Ser Leu Gly Asn Val Ile Met Val Cys Arg Lys | 595 | 600 | 605 |
| Gly Glu Trp Val Ala Leu Asn Pro Leu Arg Lys Cys Gln Lys Arg Pro | 610 | 615 | 620 |
| Cys Gly His Pro Gly Asp Thr Pro Phe Gly Thr Phe Thr Leu Thr Gly | 625 | 630 | 635 |
| Gly Asn Val Phe Glu Tyr Gly Val Lys Ala Val Tyr Thr Cys Asn Glu | 645 | 650 | 655 |
| Gly Tyr Gln Leu Leu Gly Glu Ile Asn Tyr Arg Glu Cys Asp Thr Asp | 660 | 665 | 670 |
| Gly Trp Thr Asn Asp Ile Pro Ile Cys Glu Val Val Lys Cys Leu Pro | 675 | 680 | 685 |
| Val Thr Ala Pro Glu Asn Gly Lys Ile Val Ser Ser Ala Met Glu Pro | 690 | 695 | 700 |
| Asp Arg Glu Tyr His Phe Gly Gln Ala Val Arg Phe Val Cys Asn Ser | 705 | 710 | 715 |
| Gly Tyr Lys Ile Glu Gly Asp Glu Glu Met His Cys Ser Asp Asp Gly | 725 | 730 | 735 |
| Phe Trp Ser Lys Glu Lys Pro Lys Cys Val Glu Ile Ser Cys Lys Ser | 740 | 745 | 750 |
| Pro Asp Val Ile Asn Gly Ser Pro Ile Ser Gln Lys Ile Ile Tyr Lys | 755 | 760 | 765 |
| Glu Asn Glu Arg Phe Gln Tyr Lys Cys Asn Met Gly Tyr Glu Tyr Ser | 770 | 775 | 780 |
| Glu Arg Gly Asp Ala Val Cys Thr Glu Ser Gly Trp Arg Pro Leu Pro | 785 | 790 | 795 |
| Ser Cys Glu Glu Lys Ser Cys Asp Asn Pro Tyr Ile Pro Asn Gly Asp | 805 | 810 | 815 |
| Tyr Ser Pro Leu Arg Ile Lys His Arg Thr Gly Asp Glu Ile Thr Tyr | 820 | 825 | 830 |
| Gln Cys Arg Asn Gly Phe Tyr Pro Ala Thr Arg Gly Asn Thr Ala Lys | 835 | 840 | 845 |
| Cys Thr Ser Thr Gly Trp Ile Pro Ala Pro Arg Cys Thr Leu Lys | 850 | 855 | 860 |

<210> SEQ ID NO 24
 <211> LENGTH: 2665
 <212> TYPE: DNA
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 24

| | |
|---|-----|
| cgccgccacc atgggcgag caggcttggt gggcgtgttc ctggcattgg tggcaccg | 60 |
| cgtattgggc atttcatgcg gctctctcc acccattctc aatggaagga tctctacta | 120 |
| cagcaccccc atagctgtcg gcaccgttat ccgatacagt tgttccgta ctttccggt | 180 |
| tatcggcgaa aagtctttgc tgtgcattac caaggataaa gtggacggga cttgggaaa | 240 |
| accgcacct aagtgcgagt attttaaaa atatagcagc tgcctgagc ctatagtacc | 300 |
| cggggggtat aaaatccggg gctctactcc ctatcgteat ggcgattctg tgaccttcgc | 360 |

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| | | | | | | |
|------------|------------|------------|------------|------------|-------------|------|
| atgtaaaact | aatTTTTcaa | tgaatggcaa | caagtctgta | tgggtgcaag | caaataacat | 420 |
| gtggggacct | acccgcctgc | caacctgtgt | gtcagtgttt | cccctggaat | gtccagccct | 480 |
| ccctatgatc | cacaacggac | atcacaccag | cgaaaacggt | ggatccatcg | caccagggct | 540 |
| ctctgtgact | tactcttgcg | agtccgggta | cctgctcgtg | ggtgaaaaga | tcatcaactg | 600 |
| cctcagtagt | ggtaaatggt | ccgccgtgcc | tcccacatgt | gaagaggccc | ggtgcaagag | 660 |
| cctgggccgg | ttccccaacg | gaaaagtgaa | ggaacctcct | atcttgaggg | ttgggtgac | 720 |
| cgtaacttt | ttctgcgacg | aggggtacag | gtccaaggg | cctcccteta | gtcgggtcgt | 780 |
| aatcgccggt | caaggagtcg | catggactaa | gatgcctgtg | tgtgaggaga | ttttcgagga | 840 |
| ttgtaatgaa | ttgccacca | ggagaaatac | tgaaatcctg | acaggctctt | ggctctgatca | 900 |
| gacttatcca | gaaggcacc | agggcattta | caagtgtcgg | cctggataca | gatctctggg | 960 |
| aatgtgatc | atggatgta | ggaaaggaga | gtgggtggct | ttgaaccccc | tccgcaagtg | 1020 |
| tcagaaaaga | ccatgcgggc | atcctggaga | cacccattc | gggacattta | cactgacagg | 1080 |
| cggaaacgta | tttgagtacg | gagtcaaggc | cgtttataca | tgtaacgaag | ggatcaact | 1140 |
| gctgggagaa | atcaactata | gggagtgcga | cactgacgga | tggacaaacg | acattccaat | 1200 |
| ctgcgaagtg | gtgaaatgtc | ttccagttac | agccccgaa | aacgggaaaa | tcgtgtcctc | 1260 |
| cgctatggag | cctgaccggg | aatatcattt | cgccaggcc | gtagattcgt | tgtgtaatag | 1320 |
| cggctacaaa | atcgagggcg | acgaagaaat | gcattgcagc | gatgacgggt | tctggagcaa | 1380 |
| ggagaagcct | aatgcgtcgt | aaatttcatg | caagagtccc | gacgtcataa | acggttctcc | 1440 |
| aatttcccag | aagatcattt | ataaggagaa | tgagcggttc | cagtataagt | gtaatatggg | 1500 |
| ctacgagtac | agcgaacgcg | gtgacgcegt | gtgtaccgaa | agtggctgga | gaccactgcc | 1560 |
| tagttgcgag | gagaaatcct | gcgacaacce | ttatattccc | aacggggact | actctcctct | 1620 |
| gagaatcaag | catcggactg | gcgacgagat | tacttaccaa | tgcaggaacg | gattctatcc | 1680 |
| agcaactcgg | ggcaataccg | ctaagtgtac | ctccacaggc | tggatacccg | ctcctagatg | 1740 |
| tacagaggac | tgcaatgaac | tgccacctcg | gcgcaataca | gaaatTTTga | ctggatcatg | 1800 |
| gtctgaccag | acttaccctg | agggcaccca | ggccatctac | aaatgtaggc | ccggttatcg | 1860 |
| aagtttgggt | aacgtgatta | tgggtgtcgt | aaaaggTgaa | tgggtagcac | tcaatcccct | 1920 |
| ccgtaaatgc | cagaagcgtc | cttgtgggca | cccaggcgat | accctttttg | gaactttcac | 1980 |
| cctgactgga | ggaaacgtct | ttgaatatgg | tgtgaaagcc | gtgtacacat | gcaatgaagg | 2040 |
| gtaccaactg | ctcggagaga | taaaactatc | ggagtgcgat | acagatggat | ggaccaatga | 2100 |
| tataccaatc | tgcgaggtgg | tgaagtgtct | cccagtcacc | gctcctgaga | acggaaagat | 2160 |
| cgtcagttct | gctatggaac | ctgacagggg | ataccacttt | gggcaagccg | tccgcttcgt | 2220 |
| gtgcaattca | gggtacaaga | tagaaggcga | cgaagagatg | cactgttccg | acgatggttt | 2280 |
| ctggctctaa | gagaagccta | aatgtgtcga | gattagctgc | aagtctcccg | atgttattaa | 2340 |
| cggtctctcc | atctctcaaa | aaattattta | taaggaaaac | gaaagatttc | agtacaagtg | 2400 |
| caatatgggt | tatgagtaca | gtgaacgtgg | agacgccgtg | tgcacagagt | ccgggtggcg | 2460 |
| tccactgccc | agctgcgaag | aaaaatcctg | tgacaacccc | tacatcccca | atggcgacta | 2520 |
| ttccccctcg | cgcatcaaac | atcgtactgg | cgatgaaatt | acttaccagt | gccgcaacgg | 2580 |
| gttctaccct | gccaccggg | gtaacacagc | caaatgcacc | tccaccggat | ggatccccgc | 2640 |
| cccacgctgt | accttgaat | gatga | | | | 2665 |

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<210> SEQ ID NO 25
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: signal peptide

<400> SEQUENCE: 25

Met Gly Ala Ala Gly Leu Leu Gly Val Phe Leu Ala Leu Val Ala Pro
1           5           10           15

Gly Val Leu Gly
                20

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<210> SEQ ID NO 26
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(60)
<223> OTHER INFORMATION: signal peptide

<400> SEQUENCE: 26

atgggagaccg ctggtctgct eggcgtgttc ctgccttgg tggacactgg cgctctgggc 60

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What is claimed is:

1. A pharmaceutical composition comprising a comple- 30
ment receptor 2 (CR2)-factor H (FH) molecule comprising:

a) a CR2 portion comprising a CR2 or a fragment thereof,
and

b) a FH portion comprising a FH or a fragment thereof, said 35
FH portion or fragment thereof comprising at least the
first four N-terminal short consensus repeat (SCR)
domains of FH,

wherein the CR2 portion of the CR2-FH molecule is 40
capable of binding to a CR2 ligand;

wherein the FH portion of the CR2-FH molecule is capable
of inhibiting activation of the alternative complement
pathway; and

wherein said pharmaceutical composition comprises an 45
amount of said CR2-FH molecule of about 0.01 µg/kg to
about 300 mg/kg.

2. The pharmaceutical composition of claim 1, wherein the
CR2 portion comprises at least the first two N-terminal SCR
domains of CR2.

3. The pharmaceutical composition of claim 1, wherein the 50
CR2 portion comprises at least the first four N-terminal SCR
domains of CR2.

4. The pharmaceutical composition of claim 1, wherein the
FH portion comprises at least the first five N-terminal SCR
domains of FH.

5. The pharmaceutical composition of claim 1, wherein the 55
CR2-FH molecule comprises two or more CR2 or FH por-
tions.

6. The pharmaceutical composition of claim 5, wherein the
CR2 or the FH portions are tandemly linked.

7. The pharmaceutical composition of claim 1, wherein the
CR2 portion comprises the first four N-terminal SCR
domains of CR2 and the FH portion comprises the first five
SCR domains of FH.

8. The pharmaceutical composition of claim 7, wherein the 35
CR2 portion comprises amino acids 23 to 271 of SEQ ID
NO:1 and the FH portion comprises amino acids 21 to 320 of
SEQ ID NO:2.

9. The pharmaceutical composition of claim 1, wherein the 40
CR2-FH molecule is a fusion protein.

10. The pharmaceutical composition of claim 1 further
comprising a pharmaceutically acceptable carrier.

11. The pharmaceutical composition of claim 10, wherein
the composition is suitable for intraocular, intravenous,
intraarterial, sub-cutaneous, intratracheal, or inhalational
administration.

12. The pharmaceutical composition of claim 1, wherein
the CR2-FH molecule:

a) forms dimers or multimers; or

b) comprises a derivatized CR2 or FH portion comprising
glycosylation, phosphorylation, acetylation, myristoy-
lation, prenylation, palmitation, amidation, and/or addi-
tion of glycosylphosphatidyl inositol; or

c) is fused to a compound that increases the half-life of the
CR2-FH molecule or reduces immunogenicity.

13. The pharmaceutical composition of claim 12, wherein 55
the compound is polyethylene glycol or albumin.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 9,212,212 B2
APPLICATION NO. : 14/043317
DATED : December 15, 2015
INVENTOR(S) : Gary Gilkeson et al.

Page 1 of 1

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the Claims

Column 114, Claim 12, Line 51, replace "palmitation" with --palmitoylation--.

Signed and Sealed this
Twelfth Day of July, 2016



Michelle K. Lee
Director of the United States Patent and Trademark Office